A STUDY OF THE DIGESTION AND METABOLISM OF DAIRY COWS DIFFERING IN SUSCEPTIBILITY TO BLOAT

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ABSTRACT

The digestive characteristics of cows of high (HS) or low (LS) susceptibility to bloat were investigated.

Sixteen HS and 16 LS cows were offered pasture or hay when pregnant but not lactating, and pasture or 75% pasture plus 25% hay when lactating. The susceptibility groups did not differ in voluntary intake or apparent digestibility of dry matter, organic matter, nitrogen or energy on either occasion. LS cows produced more milk of a lower protein content than HS cows but yields of fat, protein and lactose were similar for both susceptibility groups.

Subsequent experiments used 4 or 5 cows of each susceptibility group, each fitted with a fistula in the dorsal rumen and offered diets of hay or pasture.

On 3 of 8 diets offered, non-lactating LS cows had less digesta, measured by removal through the fistula, in the reticulo-rumen before feeding than did HS cows. Increasing or decreasing the quantity of digesta in the reticulo-rumen after feeding correspondingly increased or decreased both the quantity of digesta subsequently lost and the quantity remaining 21 hours after feeding, or before the next feeding, for both HS and LS cows. On all diets LS cows lost less fluid from the reticulo-rumen during feeding and more after feeding, than did HS cows. Altering the concentration of Na in rumen fluid by addition of water or electrolyte solutions did not affect the loss of fluid and DM from the reticulo-rumen of LS cows relative to that of HS cows. When offered 3 pastures differing in maturity, an increase in maturity of the pasture resulted in an increase in the quantity of digesta in the reticulo-rumen before feeding, but HS and LS cows did not differ in quantity of digesta on each of the 3 pastures. The quantity of digesta in the reticulo-rumen was greater in cows when offered a diet of low K:Na ratio than when offered a high ratio diet. On both diets, however, LS cows had less digesta in the reticulo-rumen, a lower concentration of K in plasma and excreted less K in their faeces than HS cows.

Lactating HS and LS cows offered pasture _ad libitum_ did not differ in intakes of dry matter and water, in the quantities of fluid and dry matter in the reticulo-rumen or in the rate of digestion when estimated from the loss of dry matter from nylon bags incubated in the ventral sac of the rumen.

HS and LS cows did not differ in capacity of the reticulo-rumen. The rumen fluid dilution rate, estimated using Cr EDTA, was similar for both susceptibility groups in 3 experiments and higher for LS than HS cows in one experiment.

Overall, these studies revealed few differences between HS and LS cows in the parameters measured, except that under some experimental conditions LS cows had less digesta in the reticulo-rumen than did HS cows.

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INTRODUCTION

Bloat in cattle arises when fermentation gases produced in the reticulo-rumen are trapped in a stable, persistent foam that prevents their removal by eructation. Death results from asphyxiation due to increased intraruminal pressure, and can occur within an hour of the animal commencing to feed.

The problem is widespread throughout dairying areas of New Zealand. It is typically associated with immature, rapidly growing pastures, especially those containing lucerne, red or white clover. The incidence of bloat reflects the seasonal growth patterns of pasture, being predominant in spring and autumn, though bloat can occur throughout the year, on mature pastures, and on pastures containing no legumes (Clarke & Reid 1974).

In New Zealand, bloat occurs mainly in dairy cattle, although beef cattle, and more rarely sheep, all suffer from the disorder. The most recent survey of the deaths from bloat in dairy cows (Farm Production Report 1983-84), taken during a season when bloat was not severe, found that deaths occurred in 30% of herds. In these herds, mortality due to bloat was 1.1%, equivalent to 0.4% of all cows surveyed. In years of more severe outbreaks, and particularly on individual farms, the percentage of cows lost can be considerably higher.

The costs of bloat are high. They include lost production from dead cows, cost of replacement cows, production losses arising from reduced food intake during outbreaks of bloat, costs of prevention and treatment of bloated animals, and the increased strain on farmers.

Animals vary greatly in their susceptibility to bloat, with at least part of this variation being inherited. In 1972 the Department of Scientific and Industrial Research, the Ministry of Agriculture and

Fisheries and the New Zealand Dairy Board established at the Ruakura Animal Research Station a selection and breeding project to identify and breed animals of high (HS) and low (LS) susceptibility to bloat. The cows used in the studies described in this thesis resulted from this breeding programme.

Factors contributing to animal susceptibility are poorly defined. The hypotheses that have been proposed regarding the causes of bloat, including those which attempt to explain variation in susceptibility, are reviewed in Chapter 1.1. This thesis investigates one of these hypotheses, that cows of low susceptibility have less digesta in the reticulo-rumen than do cows of high susceptibility (Cockrem _et al_. 1983, Chapter 1.1.10). Thus the factors affecting the quantity of digesta in the reticulo-rumen are also reviewed (Chapter 1.2).

Work reported in Chapter 2 describes studies on the intake, milk production and digestive efficiency of cows differing in susceptibility to bloat. Subsequent chapters describe studies with cows fistulated in the dorsal rumen. Measurements of the quantity and composition of digesta in the reticulo-rumen of non-lactating and lactating HS and LS cows are reported in Chapter 3. Chapter 4 investigates the effects on the quantity of digesta in the reticulo-rumen before feeding of 2 factors, the quantity of digesta after feeding and the maturity of the pasture. Chapters 5 and 6 describe experiments which investigated the role of Na and K in affecting outflow of digesta from the reticulo-rumen, and the effect of the K:Na ratio of the diet on the quantity of digesta and on mineral metabolism of the HS and LS cows. The final chapter discusses the results of the experiments and their implications.

CHAPTER 1

REVIEW OF LITERATURE

1.1 **Pasture bloat in cattle**

1.1.1 Introduction

This section reviews bloat in cattle. Particular emphasis is given to factors contributing to the susceptibility of animals to bloat.

Bloat is a disorder of ruminants caused by retention of gas in the reticulo-rumen. Gas produced in the reticulo-rumen by microbial fermentation and acidification of bicarbonate is normally removed by absorption through the rumen wall, passage via the omasum, and passage via the oesophagus (eructation). Bloat results when the fermentation gases are trapped in a stable, persistent foam and not removed by eructation. Death from interference of respiration by the inflated stomach can occur within an hour of the commencement of feeding.

Two forms of bloat are recognised: (1) Pasture bloat, which occurs on immature pasture, particularly that containing legumes; (2) Feedlot bloat, which occurs in animals fed diets high in grain and low in roughage.

Both forms result from excessive foaming of ruminal digesta but whereas in pasture bloat the primary foaming agents are derived from plants (Clarke & Reid 1974), in feedlot bloat the major source of the foaming agent is bacterial slime (Bartley *et al*. 1975). This review is primarily concerned with pasture bloat.

The extensive literature on bloat in cattle has been reviewed in detail by Cole & Boda (1960), Leng & McWilliam (1974) and Clarke & Reid (1974). It is generally recognised that bloat arises from an interaction between three contributing factors: animal, plant, and microbial. Most of the research on bloat has studied one causative agent without concomitant study of the others. As a consequence, there is an incomplete understanding of the interactions between these factors. Difficulties in studying bloat arise from its sporadic occurrence. An important factor is animal susceptibility, yet many workers have failed to identify the susceptibility of their experimental animals, or have compared days on which bloat occurred with days when no bloat occurred for the same animal. Clarke & Reid (1970) have emphasised the importance of establishing the susceptibility of experimental animals since factors which limit bloat in animals of differing susceptibility may not be the same as those limiting bloat in animals of the same susceptibility. Consideration is given here to the extent that animals vary in susceptibility to bloat and to hypotheses about the cause of bloat, particularly those that attempt to account for the animal variation.

1.1.2 Susceptibility

It has long been recognised that animals vary in their susceptibility to bloat (Hancock 1954; Johns 1954; Ferguson & Terry 1955; Hungate _et al_. 1955; Johns _et al_. 1958; Cole & Boda 1960; Reid 1960; Mendel & Boda 1961; Meyer _et al_. 1964; Reid _et al_. 1972; Clarke & Reid 1974). Similarities have been observed in bloating behaviour within twin pairs compared with that between twin pairs (Hancock 1954; Johns 1954; Cockrem & McIntosh 1976), and Reid _et al_. (1972) identified sires which left larger proportions of less susceptible progeny than did others. These observations suggested a genetic basis for the variation in susceptibility between animals.

Reid _et al_. (1972) suggested that investigation of factors contributing to differences in susceptibility would provide a rational basis for a breeding project. Johns (1954) described a grading system

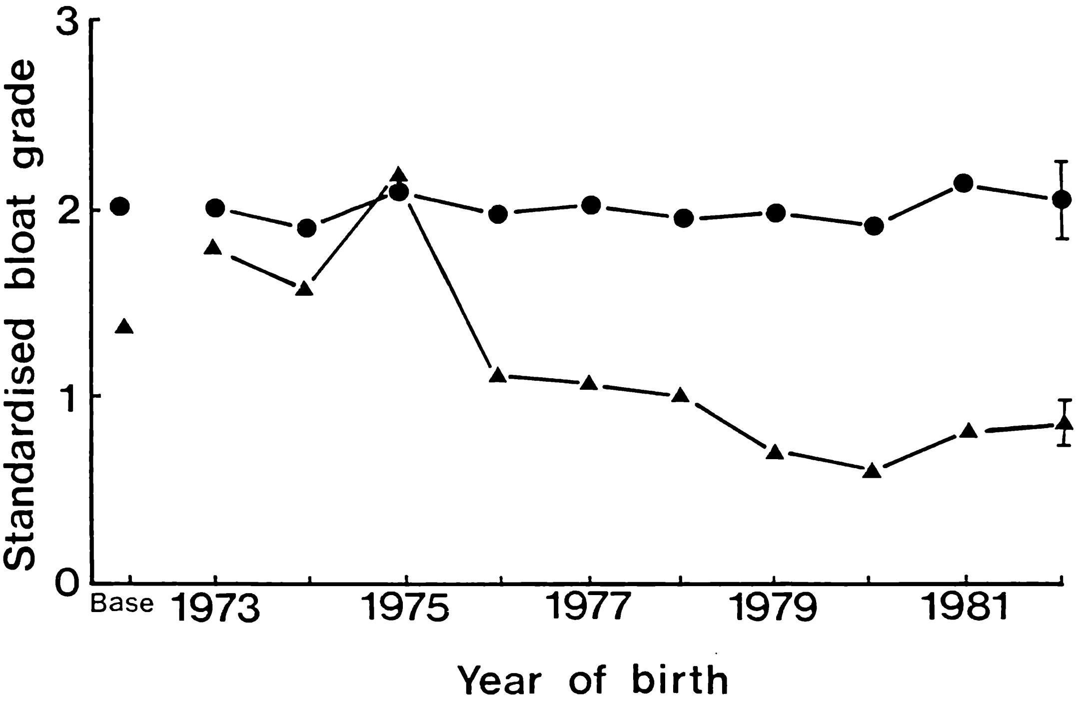


Figure 1.1 The effect of breeding and selection for high (●) and low (▲) susceptibility to bloat on the bloat grade (standardised between years) of cows born from 1973 to 1982 (unpublished data, F.R.M. Cockrem & J.T. McIntosh). Also shown are the standard errors of the means for cows born in 1982.

ranging from 0 (no bloat) to 4 (severe bloat requiring treatment to prevent death) which assessed the degree of bloat by palpation of the flanks as well as visual grading. This system has subsequently been used, except that palpation has often been omitted for ease of scoring grazing animals. A selection and breeding programme with dairy cows was started at Ruakura in 1972. Cows graded for bloat and designated to be of either high (HS) or low (LS) susceptibility, were mated to Dairy Board Jersey and Friesian bulls of the appropriate susceptibility (estimated from progeny test reports) from 1972 to 1976, and subsequently to bulls bred from the HS and LS herds. The progress made by this breeding programme is shown in Figure 1.1. Heritability of susceptibility was estimated to be about 0.6 (Cockrem 1975). Cockrem & McIntosh (1976) showed that there was an interaction between the bloat potency of the pasture and variation in susceptibility, with full animal variation in susceptibility only evident when the pasture potency was such that the mean bloat grade of the herd was above 2. Work to investigate possible genetic markers in the HS and LS cows was started by Clarke _et al_. (1974), who suggested that a salivary protein, designated band 4, may be a suitable marker. Cockrem (1975) postulated how differences between HS and LS cows in the quantity and composition of saliva may be the result of gene action. Progress in describing relationships between protein bands in saliva, saliva flow rate and susceptibility has been reported by McIntosh (1975, 1978), Reid _et al_. (1975), McIntosh & Cockrem (1977), and Cockrem _et al_. (1983), and is discussed in more detail in Section 1.1.8.

The factors contributing to susceptibility have yet to be identified (Clarke & Reid 1974). They may include salivary volume and composition, fluid transfer from the rumen, reticulo-rumen motility and eructation efficiency, gas production, microbial population, rate of eating, rumen pH and breakdown products of metabolism (Mendel & Boda 1961). Clarke & Reid (1974) added to this list the particulate matter turnover, effective sensitivity of nerve endings of the gut wall, rumen epithelium permeability, endocrine activity, and effectiveness of acid-base regulation. Degree of chewing and release of soluble protein has also been suggested (Bryant 1964), although Johns (1954) reported that the rate of food ingestion was not important. There are inadequate data available to evaluate conclusively all of these factors. Those that have been investigated are discussed below.

1.1.3 Stable foam formation

Of the many hypotheses proposed regarding the cause of bloat, formation of foam in the reticulo-rumen has been regarded as the most significant. The importance of stable foam formation in the reticulo-rumen has long been recognised (Quin 1943; Clark 1948; Reid 1960; Clarke & Reid 1974). The physical characteristics of foam stability and persistence have been investigated by Laby (1969, 1975) and Buckingham (1970). Although characteristics of foams are critical to bloat, their discussion is considered to be outside the scope of this review.

1.1.3.1 Foaming agents

Proteins in plants, saliva and of microbial origin are known to be the major foaming agents in the digesta in the reticulo-rumen. Proteins which reach the surface of the rumen fluid without being degraded by proteolysis may be denatured and these surface-denatured proteins stabilise the foam. In addition to proteins, pectins and saponins have been implicated in producing foams.

Plant proteins

Mangan (1959) provided evidence of the involvement of pasture proteins in bloat when he demonstrated that the maximum foam strength of the cytoplasmic proteins of red clover (*Trifolium pratense* L.) occurred between pH 5.4 and 6.0. Foam strength depended on the salt concentration. The strength of foams generated from centrifuged rumen fluid also showed an optimum pH of between 5.4 and 5.7, similar to that of cytoplasmic protein.

Many correlations have subsequently been reported between the incidence of bloat on lucerne (*Medicago sativa* L.) and a range of plant nitrogenous or protein components including plant nitrogen content (Miltimore *et al*. 1964), soluble nitrogen and soluble protein nitrogen (Howarth *et al*. 1977a), and total and soluble leaf chloroplast protein levels (Stifel *et al*. 1968b). McArthur *et al*. (1964) identified an 18S protein, or Fraction 1 protein, as a cause of pasture bloat. Fraction I protein is the major soluble chloroplast protein and can account for up to 50% of total soluble protein in leaf tissue (Ellis 1978). Other chloroplast proteins found in smaller quantities have been grouped as Fraction II proteins. McArthur & Miltimore (1966) concluded that the bloat-causing legumes lucerne and red clover have higher amounts of Fraction I protein, expressed as a percent of dry weight and as a percent of total protein, than either birdsfoot trefoil (*Lotus corniculatus* L.) or orchard grass (*Dactylis glomerata* L.), neither of which are known to cause bloat. Miltimore *et al*. (1970) concluded that a threshold level of 1.8% Fraction 1 protein was required for bloat to occur, although their correlations between bloat and plant constituents were improved by grouping the data into classes and thereby removing a proportion of the experimental variation.

In vitro studies have shown that Fraction 1 protein produces foams of maximum persistence, rigidity and surface denaturation at pH values close to 5.8, and that Fraction II proteins produce persistent foams over the pH range 4.4 to 6.3 (Jones & Lyttleton 1969, 1972a). Howarth *et al*. (1977b) concluded from a consideration of protein characteristics that both Fraction I and the majority of Fraction II proteins would be expected to produce viscous foams at the pH of reticulo-rumen digesta found in cattle consuming fresh lucerne. Howarth *et al*. (1977a) reported a better relationship between bloat occurrence and the presence of Fraction II proteins, than between bloat and Fraction I protein.

Stifel *et al*. (1968b) suggested that protein was synthesised in preference to chlorophyll in chloroplasts of lucerne during stages when the lucerne caused bloat. Jones & Lyttleton (1969) considered that the results of Stifel *et al*. (1968b) should be treated with caution because their recovery of protein in the chloroplast fractions was low. Stifel *et al*. (1968a) have also found a direct relationship between bloat and the extent and strength of binding of calcium and magnesium to Fraction I protein. They suggested that the binding enhanced protein denaturation, thereby increasing foaming potential.

Nugent *et al*. (1983) showed that the rate of proteolysis of plant proteins in rumen fluid depended on the diet and on the presence of other proteins. Rates were higher on lucerne than on hay and concentrates, but addition of bovine serum albumin to Fraction I protein reduced the rates of proteolysis of both proteins, compared to the rates obtained when each protein was alone in the rumen fluid. Thus the presence of a number of proteins in the reticulo-rumen could slow down or alter the pattern of breakdown of proteins which produce foams.

Salivary mucoprotein

Saliva is produced from a number of glands and its composition varies according to its origin and flow rate. It has been classified (see Kay 1960) as:

1. Serous - a thin, watery secretion which is isotonic with blood, high in bicarbonate, and low in protein and mucin. Serous saliva is produced continuously from the parotid and inferior molar glands at varying rates in response to stimulation of the mouth, oesophagus and reticulo-rumen.
2. Mucous - a thick secretion which is hypotonic to or isotonic with blood and contains bicarbonate, mucoprotein and mucin. It is produced from the palatine, buccal and pharyngeal glands in response to oral, oesophageal and reticulo-ruminal stimulation during feeding.
3. Mixed mucous + serous - a hypotonic and weakly buffered secretion low in bicarbonate and phosphate. It is produced by mandibular, sublingual and labial glands in response to feeding, but not in response to stimulation of the oesophagus or reticulo-rumen.

Mangan (1959) found that the mucoprotein of saliva aspirated from under the tongue of cattle was a powerful foaming agent. Jones & Lyttleton (1972b) and Jones _et al_. (1978) demonstrated that salivary mucoprotein, thought to have originated from the mandibular and sublingual glands (Jones & Lyttleton 1972b) produced persistent foams of low compressive strength, unaffected by pH over the range 4.0 to 7.0. Jones & Lyttleton (1973) also isolated a glycoprotein from rumen fluid which contributed to foaming. Antibodies prepared against this glycoprotein cross-reacted to desialated salivary mucoprotein, suggesting that salivary mucoprotein had foaming properties before and after desialation by microbial mucinolytic activity.

Nugent et al. (1983) suggested that mandibular protein may behave like bovine serum albumin, which slows the rate of proteolysis of Fraction I protein in the reticulo-rumen, possibly resulting in more protein available for foaming.

Microbial proteins

Clarke (1965b,c) obtained a relationship between the weight (size rather than number) of protozoa and bloat score, and suggested that the bursting of holotrich protozoa and the release of cell contents, including proteins, could contribute to foam formation. Holotrich protozoa are known to store sugars as starch, and to subsequently burst (Clarke 1965a). Jones & Lyttleton (1972b) found that holotrich protozoal proteins were denatured at the air-surface interface and produced foams of high compressive strength with maximum persistence in the pH range 5.5 to 6.5.

Clarke et al. (1969) showed that anti-protozoal agents such as dimetridazole reduced the incidence of bloat but did not prevent it entirely. The dimetridazole treatment markedly reduced and sometimes eliminated the protozoal population (Clarke & Reid 1969). Thus the presence of holotrich protozoa is not necessary for bloat to occur, but may be a contributing factor. The host animal could influence protozoal numbers through eating and drinking patterns, salivary composition and flow, rumen fluid volumes, and rates of flow of digesta from the reticulo-rumen (Hume 1974). Leng (1974) speculated that LS animals may be incapable of maintaining a stable pH in digesta, resulting in a low protozoal population.

Pectins

Conrad et al. (1958) proposed that pectins were capable of contributing to stable foams. Wright (1961) suggested that a high degree of esterification of pectin would result in a ready availability of calcium and sodium ions, which can influence foam stability (Mangan 1959). However, Wright (1961) was unable to demonstrate any differences in either the pectin content (up to 6.6% in red clover), or its degree of esterification, between bloat-causing and non bloat-causing clovers.

Clarke & Reid (1974) concluded that pectins are not a primary foaming agent, but it is currently thought that they may increase rumen fluid viscosity and act as foam stabilising agents (Jones *et al*. 1978).

Saponins

Although saponins have foaming ability through possession of surface active or detergent properties, and can exert inhibitory effects on smooth muscle activity (Cheeke 1971), they are not considered to play a role in the genesis of bloat foams (Mangan 1959) or the onset of bloat (Clarke & Reid 1974; Majak *et al*. 1980).

1.1.3.2 Anti-foaming agents

Lipids

Chloroplast lipids present in the particulate fraction of digesta have been attributed with anti-foaming properties (Mangan 1959), altering the quality of the foams rather than the volume of foam produced. Jones & Lyttleton (1973) found the concentration of lipids in foams to be 2-2.5 times that occurring in rumen fluid. They found that foams derived from centrifuged and uncentrifuged rumen fluids possessed different compressive strength and persistence properties, and suggested that lipids may exert an effect on foam persistence by spreading from the solid particles on to the surface films of the bubbles. Stifel *et al*. (1968b) found the chloroplast lipid level to be inversely related to bloat incidence and suggested that synthesis of lipids, in preference to protein, occurred during periods when the forage did not cause bloat. Stifel *et al.* (1968a) proposed that lipids competed with proteins for metal ions and thus reduced the foam-stabilising effect of metal and protein binding.

The full role of lipids in bloat is not yet clear. A nominal dose of 80 g vegetable or paraffin oil will prevent bloat when the daily intake of pasture lipids is 5 times this amount (Clarke & Reid 1974), suggesting that the form of the lipid is important to its effect on bloat.

Tannins

The presence of condensed tannins has been associated with legumes which have not been shown to cause bloat. Tannins form insoluble complexes with leaf protein, preventing foaming (Kendell 1966). Inhibition of foaming by tannins can be reversed by addition of polyvinylpyrrolidone (PVP), a reagent which binds tannins (Kendell 1966; Jones *et al.* 1970). Jones *et al.* (1970) showed that leaf extracts from lotus (*Lotus pedunculatus* Cav.) released no soluble protein compared with red, white (*Trifolium repens* L.) and subterranean (*T. subterraneum* L.) clovers, unless PVP was added to the extracting medium. Barry & Forss (1983) found that the minimum concentration of condensed tannins necessary to precipitate protein was 2-4% of the dry matter. Condensed tannins effectively precipitated protein in mixtures of lotus and red or white clovers (Jones *et al.* 1970; Barry & Forss 1983). Jones (1971, cited by Clarke & Reid 1974) found that condensed tannins of lotus precipitated leaf protein, rumen protozoal proteins, salivary mucoprotein and foaming agents in rumen fluid from bloating animals. Condensed tannins from sainfoin (*Onobrychis viciifolia* Scop.) were found to form complexes with Fraction I protein from lucerne but not with bovine mandibular mucoprotein at physiological temperatures (Jones & Mangan 1977).

Attempts to find tannins in leaf laminae of white or red clover (Jones et al. 1973) or Medicago species (Goplen et al. 1980) have been unsuccessful, therefore the possibilities of breeding clovers and lucerne which do not cause bloat are low unless gene transfer techniques are applicable. Results obtained by Dalrymple et al. (1984) indicated that a single gene is responsible for tannin production in birdsfoot trefoil.

Animal mucins

The addition of bovine saliva or plant mucin to foaming reticulo-rumen contents increased the rate of release of trapped gas from the foam (Van Horn & Bartley 1961). Bartley & Yadava (1961) suggested that salivary mucin acted as a foam inhibiting and foam breaking agent and that bloat resulted when feeds containing foaming constituents failed to induce sufficient salivary secretion. Fina et al. (1961) isolated 5 mucinolytic organisms in the reticulo-rumen which, when introduced into the reticulo-rumens of non-bloating cows, caused bloat. Mishra et al. (1967, 1968) found that mucinolytic bacteria in the reticulo-rumen degraded salivary mucin, and they postulated that mucinolytic activity destroyed the anti-foaming activity of mucin. Mishra et al. (1967) showed differences in the number of mucinolytic organisms between twin mates on hay and pasture (non-bloating) and those on pasture only (bloating). However, they did not include in their comparison animals consuming pasture only and not bloating, therefore the association between mucinolytic organisms and bloat was not conclusive.

Subsequently, Jones & Lyttleton (1972b) demonstrated that salivary mucin collected from the oesophagus of cows produced a viscous solution but did not produce a persistent foam. They suggested (Jones & Lyttleton 1973) that mucinolytic activity produced a foam stabilising molecule from mucoprotein rather than destroyed an anti-foaming agent.

The full role of mucins has yet to be determined.

1.1.4 **Plant morphology**

Howarth et al. (1978) proposed that leaf cell rupture and the ensuing release of intracellular constituents are important factors affecting the bloat potency of fresh herbage. Species studied included sainfoin, cicer milkvetch (Astragalus cicer L.), and birdsfoot trefoil, which are not known to cause bloat, and lucerne, and red and white clover, which cause bloat. Extensive studies on plant morphology (Howarth et al. 1978; Lees et al. 1981, 1982), and microbial invasion (Fay et al. 1980, 1981; Howarth et al. 1982) or enzymic digestion (Sant & Wilson 1982) of leaflets of these legumes indicated that those legumes which did not cause bloat had epidermal and mesophyll cells which were more resistant to mechanical rupture, and therefore less disrupted by chewing, than those that caused bloat. These differences between species affected their rate of digestion and a nylon bag incubation technique was used to determine the initial rate of digestion (IRD) of herbage types (Howarth et al. 1982). It was estimated that a 25-30% reduction in IRD was required in order to reduce the bloat potency of lucerne, and a plant selection programme was initiated to achieve this (Goplen et al. 1982). Kudo et al. (1985) offered sheep two selected strains of lucerne which differed in IRD by 6%, and found higher concentrations of chlorophyll, soluble protein, soluble carbohydrate, and volatile fatty acids in rumen fluid from sheep when offered the fast IRD strain than when offered the slow IRD strain.

The work on plant morphology is based on the assumption that cell strength is related to bloat potential. It does not explain the variation between days in bloat incidence. This deficiency may be clarified by studies on the chewing and digestion of strains of lucerne differing in IRD, by cattle of known susceptibilities to bloat.

1.1.5 **Concentrations of minerals in herbage**

Attempts have been made to link the incidence of bloat with plant factors such as mineral concentration. Data on the effects of fertilisers and soil mineral levels on bloat incidence are confusing and often contradictory (Clarke & Reid 1974). Generally, management factors which increase sward vigour or the proportion of legumes increase the incidence of bloat. Concentrations of minerals in herbage change with changes in plant maturity (Fleming & Murphy 1968), season (Metson & Saunders 1978) and species (Smith et àl. 1978), therefore it is inevitable that correlations will occur between the concentrations of some minerals and the incidence of bloat. Such correlations do not prove a causative relationship. Correlations of the mineral concentrations of legumes and the incidence of bloat have been inconsistent (Clarke & Reid 1974). For example, Miltimore et al. (1970) reported positive correlations between bloat and the concentrations of calcium, nickel and zinc; the calcium concentration was positively associated with the Fraction I protein concentration. No relationship could be demonstrated between bloat and the concentration of magnesium. In contrast Stifel et al. (1968b) reported a positive correlation between bloat and magnesium concentration of chloroplasts and a negative correlation between calcium concentration and bloat. Miltimore et al. (1964) reported no relationships between bloat incidence and concentrations of phosphorus, potassium, sulphur, calcium or magnesium in lucerne.

Bloat may occur on the same pasture as that which causes grass tetany (Stewart _et al_. 1981), the latter possibly being associated with the herbage potassium:(calcium + magnesium) ratio (Allcroft & Burns 1968). Turner (1981) surveyed 12 farms and suggested that farms with a high incidence of bloat had high herbage potassium:sodium ratios, compared with those with a low bloat incidence.

Cheng _et al_. (1979) reported that the addition of NaCl to a concentrate diet reduced the incidence of feedlot bloat, through an increase in outflow and turnover of digesta in the reticulo-rumen and a reduction in lysis of bacterial cells. There are no published data available on the effect of mineral supplements on the incidence of pasture bloat.

1.1.6 Gas production in the reticulo-rumen

There have been several attempts to relate the bloating behaviour of animals to the gas production of their rumen fluid. Hungate _et al_. (1955) found that the rate of gas production of rumen fluid from bloating steers was higher than that from non-bloating steers. However, control animals were those not bloating on any given day and therefore they took no account of phenotypically LS steers and those not bloating for other reasons. They also did not record reticulo-rumen dry matter content, total or protein nitrogen, microbial numbers, or other bases for comparison between bloating and control animals (Walker 1974). Other studies have found no differences in gas production between bloating and control animals (Mendel & Boda 1961; Clarke & Hungate 1971; Cockrem _et al_. 1983). Majak _et al_. (1983) concluded that the rates of gas production from rumen fluid obtained before feeding and with added glucose, were higher in cattle that subsequently bloated than in those that did not. However, these workers also compared animals which bloated with the same animals when not bloated. Many of the studies on fermentation rates, including those of Clarke & Hungate (1971), Cockrem _et al_. (1983) and Majak _et al_. (1983) used strained rumen fluid. It is well established that the microbial activity and gas production of rumen fluid differs from that of whole rumen contents (Forsberg & Lam 1977; Fay _et al_. 1980). In addition, the presence of particulate matter affects the characteristics of the foam. Mangan (1959) showed that particulate matter had anti-foaming properties and Jones & Lyttleton (1973) found that foam derived from centrifuged fluid had higher compressive strength and lower persistence than that from uncentrifuged fluid.

Clarke & Reid (1974) concluded from reviewing the literature that if there were microbial differences between bloating and non-bloating animals or between animals of different susceptibilities to bloat, then the differences were not reflected in the end products of digestion, that is, gases, volatile fatty acids and nitrogenous compounds, or a change in pH. This conclusion must be regarded as tentative until clarified by further studies on whole reticulo-rumen contents from cows differing in susceptibility.

1.1.7 Physical deficiency of the diet

Mead _et al_. (1944) proposed that bloat was due to a physical deficiency of the diet, and that diets which caused bloat contained insufficient roughage to stimulate eructation. Eructation occurs during the second of the two regular cyclical sequences of contraction that occur in the reticulo-rumen. The first (primary or A sequence) consists of a diphasic contraction of the reticulum, followed by a contraction of the rumen, and is primarily concerned with mixing.

The second (secondary or B sequence) is independent of the reticulum and only involves the rumen. This is also known as the eructation sequence. Contractions are responses to medullary reflexes, influenced by stimuli originating in the rumen and reticulum. Motility of the reticulo-rumen has been well reviewed (Stevens & Sellers 1959; Reid 1963; Sellers & Stevens 1966; Titchen 1968; Iggo & Leek 1970; Leek & Harding 1975).

The process of eructation and its importance in clearing fermentation gases, in relation to bloat, was reviewed by Clarke & Reid (1974). There is little evidence to support the physical deficiency hypothesis. For example, Johns (1954) found that the eructation reflex was more active during moderate bloat than in the normal animal. However, while it is considered that failure of eructation does not result in bloat, effects of reticulo-rumen distension in bloating animals on circulation, salivation, gastric motility and other reticulo-rumen processes are not well described. Weiss (1953b) and Mendel & Boda (1961) suggested that reticulo-rumen motility or eructation efficiency may be involved in differences between animals in susceptibility to bloat. Recently, Waghorn & Reid (1984) measured the resting levels and vertical displacement during contractions, of the cranial pillar, cardia, reticulo-omasal orifice and other structures in the reticulum and rumen of 2 HS and 2 LS cows when fed on hay or on red clover with a paraffin additive to prevent bloat. Bloat susceptibility was not correlated with rumen depth (vertical displacement between highest and lowest points in the rumen), location of the cardia, or resting level of the cranial pillar relative to rumen depth. No set of anatomical characteristics was found to typify the HS or LS animals. The frequency of the A and B sequences before feeding and the B sequence during feeding were higher for LS than for HS cows, but it was concluded that insufficient data were available to evaluate whether contraction frequency contributed to susceptibility to bloat. Waghorn & Reid (1984) noted considerable variation between days in the characteristics measured, and speculated that aspects of motility may account for some of the day-to-day variation in bloat incidence in individual cows. Studies involving more animals and including within animal comparisons of periods of bloat and no bloat are required to fully evaluate the role of motility in susceptibility.

1.1.8 The quantity and composition of saliva

Weiss (1953a) suggested that feeds which resulted in bloat did not provide sufficient stimulus for salivation, resulting in viscous, frothy reticulo-rumen contents. There are data which support this hypothesis (Meyer et al. 1964), but dietary factors such as roughage content which affect salivation also affect other aspects of rumen function, such as fermentation. It has been suggested that the quantity of saliva produced may contribute to susceptibility differences. Mendel & Boda (1961) reported that LS cows secreted 5 and 28% more saliva (collected at the cardia) during rest and feeding, respectively, than did HS cows. Meyer et al. (1964) were unable to demonstrate differences between HS and LS cows in resting saliva secretion, although their comparison was confounded by breed differences. No differences between HS and LS cows in salivation rate during feeding were reported by Reid et al. (1972). McIntosh & Cockrem (1977) and McIntosh (1978) found that LS cows secreted saliva at a faster rate than did HS cows when the saliva was collected using a perforated bit placed under the tongue. This difference in flow rate between HS and LS cows was not maintained, however, in later progeny (Cockrem et al. 1983). The reasons for this are not clear.

Lyttleton (1960) found a similarity between members of twin sets and differences between twin sets in the salivary protein pattern, suggesting an inherited characteristic in salivary composition. Clarke _et al_. (1974) found that the concentration of one protein band, designated band 4, was higher in HS cows than in LS cows, and suggested that band 4 may be a suitable genetic marker for a breeding programme to reduce bloat. Preliminary results from electrophoretic studies of salivary proteins showed several protein bands appeared to be correlated with bloat (McIntosh 1975). This work and subsequent investigations (McIntosh & Cockrem 1977; McIntosh 1978) showed a correlation between band 4 and bloat grade at the time of sampling. Band 4 was increased on grass or clover if causing bloat and was high in bloated cows whether HS or LS. Clarke _et al_. (1974) suggested that the structure of band 4 may be different between HS and LS cows because they found that saliva was degraded more readily by rumen micro-organisms from the cow supplying the saliva than by micro-organisms from other cows. However, band 4 from both HS and LS cows was found to cross-react with the same anti-serum (McIntosh 1978). Other bands, namely 6 and 7, and saliva flow rate were also correlated with bloat susceptibility (McIntosh 1975, 1978; McIntosh & Cockrem 1977). McIntosh _et al_. (1984) showed that under sympathomimetic and parasympathomimetic stimulation most of the major salivary proteins were secreted by both the parotid and mandibular glands but that quantitative differences for specific proteins existed. Most of band 4 protein was secreted from the parotid and band 7 (and 8, which is chemically related to band 7) from the mandibular gland.

Further studies on electrophoretic (McIntosh _et al_. 1982) and monoclonal antibody (Jones _et al_. 1984) techniques for studying the proteins are in progress. A suitable sampling technique for obtaining repeatable relationships between proteins and susceptibility, for use as a marker, has not yet been identified. The actual functions in the reticulo-rumen or elsewhere of the specific proteins, with respect to bloat, have also to be determined. Band 6 may be bovine serum albumin (Cockrem et al. 1983), which is more slowly degraded in the rumen than are other proteins (Nugent et al. 1983).

Mendel & Boda (1961) attempted to relate susceptibility to the concentrations of minerals in saliva, but found inconsistent differences between HS and LS cows in the concentrations of potassium, sodium, bicarbonate and phosphate in saliva.

The exchange between HS and LS animals of boli collected at the cardia during feeding did not alter bloating behaviour, suggesting that chewing and salivation are not important in the short term (Clarke & Reid 1970). Cockrem et al. (1983) found that LS cows bloated when their level of digesta in the reticulo-rumen was increased before feeding by additions from another cow. These digesta exchange experiments (Section 1.1.10) also suggested that the origin of saliva produced while bloat was occurring was not important in determining bloat behaviour. However, these experiments did not test the importance of saliva to the incidence of bloat under different pasture or environmental conditions.

1.1.9 **The composition of digesta in the reticulo-rumen**

The immediate site of action for factors determining susceptibility lies within the reticulo-rumen. When LS cows bloat, their reticulo-rumen contents are similar in appearance to those of HS cows (Reid 1960). Foam is often present in LS or non-bloating cows' digesta but it is not stable (Hungate et al. 1955; Mendel & Boda 1961), suggesting that it has foaming ability but lacks stabilisation factors. Several workers have suggested that an accumulation of some factor in the reticulo-rumen is required for bloat to occur, and that reticulo-rumen contents prior to the occurrence of bloat determine whether bloat will occur, rather than the forage consumed at the time of bloat (Ferguson & Terry 1955; Mendel & Boda 1961; Majak _et al._ 1983). Mendel & Boda (1961) suggested that LS cows may not accumulate the necessary factor(s) due to (a) more complete digestion; (b) larger entry of salts into the reticulo-rumen and therefore increased buffering capacity and prolonged fermentation; (c) gas release enhanced by larger amounts of mucin entering the reticulo-rumen; (d) lower ingesta surface tension brought about by a large volume of parotid saliva; (e) a combination of some or all of (a)-(d). Reid _et al._ (1972) reported no differences between HS and LS animals in the buffering capacity of digesta before feeding. Majak _et al._ (1983) examined dorsal sac contents before the ingestion of lucerne and found higher concentrations of chlorophyll, increased buoyancy of particulate matter, and an increased rate of gas production when glucose was added to strained rumen contents from animals which subsequently bloated than from the same animals when they did not bloat. Chlorophyll concentration was still elevated in bloating animals, compared to non-bloating, 4 hours after feeding. Majak _et al._ (1983) suggested that microbial colonisation and retention of particulate matter could provide an active inoculum for promoting rapid legume digestion, thereby enhancing fermentation but trapping the gas in the buoyant frothy digesta. Majak _et al._ (1985) reported that individual animals showed higher chlorophyll concentrations when their rumen fluid was frothy than when it was not, except that data for one animal which had the least number of frothy cases and was possibly an LS animal showed no difference in chlorophyll concentration between frothy and non-frothy rumen fluid. Whether reticulo-rumen digesta characteristics which cause day-to-day variation in the incidence of bloat in susceptible animals are similar to those which contribute to susceptibility, is not known. Majak *et al*. (1985) found that there was no relationship between the concentration of soluble protein in rumen fluid and the occurrence of frothy contents, and speculated that a component of chloroplasts was responsible for the onset of foam formation.

Cows susceptible to bloat have been found to have a higher (Mendel & Boda 1961), similar (Reid *et al*. 1972), or lower (Clarke & Reid 1974) content of dry matter in digesta than do non-bloaters. Clarke & Reid (1974) reported that LS cows' digesta, after 18 hours fasting, had an increased proportion of coarse fibrous residues and less finely divided particulate matter associated with their higher dry matter content than that of HS cows.

1.1.10 **The quantity of digesta in the reticulo-rumen**

Cockrem *et al*. (1983) suggested that the volume of reticulo-rumen contents may be an important factor in determining susceptibility. After observing that LS cows appeared to have less digesta in the reticulo-rumen after a period of fasting than did HS cows, they exchanged reticulo-rumen contents within and between 2 HS and 2 LS cows and found that bloat was associated with a greater volume of digesta, regardless of the susceptibility of the host or donor cow. The variance in bloat grade accounted for by reticulo-rumen digesta volume was 64%. It was estimated that the volumes of digesta of HS and LS cows after 12 h fasting were 40 and 30 ℓ, respectively. Cockrem *et al*. (1983) calculated from these observations *in vivo* and from gas measurements *in vitro*, that the observed HS/LS differences in bloat grades could be accounted for by the digesta volume difference observed. They speculated that aspects of fluid balance, including effects of feeding on plasma volume and osmolality, saliva flow, water exchange across the rumen wall and sodium and potassium metabolism, may be involved in genetically determining the digesta volumes.

Few data are available to evaluate this hypothesis. Clarke & Reid (1970) found that the reversal of bloating behaviour between HS and LS cows after the total exchange of reticulo-rumen contents was only temporary, lasting some 24 hours. If volume was the factor transferred then this is consistent with the observations of Cockrem _et al_. (1983) who found that volumes of digesta in the reticulo-rumen returned to pre-exchange volumes in each cow within 24 hours (Cockrem, pers. comm.). Mendel & Boda (1961) concluded that the volume and weight of reticulo-rumen digesta were not related to susceptibility, but they fed all cows at the same level of intake without providing live weight data, therefore intake relative to live weight was not available.

Work to date on water metabolism has failed to find differences between HS and LS cows. Reid _et al_. (1972) stated that no consistent differences existed between HS and LS animals with regard to water flows through the rumen, but no data were presented to support this and no information was provided on the techniques used. Wright & Jones (1974) reported that no relationship existed between body water turnover, estimated using tritiated water, and bloat incidence. However, although they classed their cows as HS and LS, all animals bloated during the trials despite a maximum score recorded of only 2.4, which suggests that pasture potency was not severe. They observed that cows dosed with a non-ionic polymer of ethylene and propylene glycols (Pluronic L-64) had a higher water turnover than did untreated twins. This could have arisen through increased water and dry matter intakes in the treated cows. Ternouth (1967) suggested, without evidence, that an accumulation of water in the rumen, due to a net flux of water into the reticulo-rumen across the epithelium, could be a causative factor in bloat.

Cockrem _et al_. (1983) reported that a negative relationship existed between blood haematocrit values and bloat susceptibility. McIntosh _et al_. (1985) reported kallikrein-like enzyme activity in secretions obtained from the mandibular gland of 3 cows after parasympathomimetic stimulation, and speculated that kinins produced may alter the permeability of the rumen wall of HS and LS cows.

1.1.11 **Conclusions**

This review is not exhaustive but has indicated the complexity of the interactions between plant, animal and microbial factors which contribute to the occurrence of bloat. Interactive effects have been overlooked in many studies and prediction of when bloat will occur is still confined to generalisations (such as pasture type) without a full understanding of the system. Two aspects have yet to be resolved: (1) what causes the variation from day-to-day in the occurrence of bloat in animals which are susceptible to bloat and (2) what are the factors contributing to the susceptibility of the animal. Many studies have been concerned with the first of these, but have often confounded the two because susceptibility of the animals was not known. Studies of animal factors have suffered from the use of too few animals, and this may be one reason why progress on genetic markers to identify susceptibility has been slow. Pasture studies attempting to identify those plant constituents responsible for bloat have often been based on correlations of daily concentrations of constituents with the incidence of bloat, without obtaining information on the effect of changes in plant composition on the reticulo-rumen environment of both HS and LS animals.

The exchange experiments of Cockrem et al. (1983) (Section 1.1.10) indicated that HS and LS cows differed in the volume of digesta in the reticulo-rumen, and that a change in digesta volume had an effect on the bloat behaviour of the cows. This thesis investigates the hypothesis that LS cows have less digesta in the reticulo-rumen than do HS cows. The investigation may not only result in a better understanding of factors contributing to susceptibility, but may also indicate a means of control of bloat through the manipulation of the quantity of digesta, and may enable the quantity of digesta in the reticulo-rumen or a related characteristic to be used as a genetic marker. Investigation of the hypothesis requires a knowledge of the inputs to and outputs from the reticulo-rumen, and their interrelationships, regulation and effects on the quantity of digesta. The following section considers these aspects.

1.2 Factors affecting the quantity of digesta in the reticulo-rumen

1.2.1 Introduction

Digestion in the ruminant is a dynamic process involving the inflow of feed, water and saliva to the reticulo-rumen, absorption of solutes and products of fermentation across the rumen wall and outflow of fluid, micro-organisms and undigested feed residues through the omasum to the lower digestive tract. Genetic control of the quantity of digesta could be exerted through regulation of the quantity or composition of any one or a combination of the inputs to and outputs from the reticulo-rumen.

Terms used in the literature and in this review to describe digesta turnover include:

Dilution rate, D, is the fraction of the component being studied that moves out per unit time. Alternative expressions for D include clearance rate or fractional outflow rate.

Flow, F, is the actual amount of the component entering and leaving the digesta pool per unit time (assuming a steady state).

Volume or mass, V, is the quantity of the component in the digesta pool.

Mean retention time, MRT, is the average time that the component of digesta is retained in the digesta pool.

These variables are related by the expressions:

$$D = \frac{F}{V} \quad \text{and} \quad MRT = \frac{1}{D}$$

Much of the following review refers to sheep, reflecting the paucity of relevant data on cattle.

1.2.2 Fluid and solid pools of digesta

Digesta consists of two major pools, the fluid pool which is taken to include soluble components and bacteria and small particles, and the solid or particulate pool, taken to include undigested and indigestible feed components. Solid particles may be caught in the mass of solid digesta, or if free, be retained by the straining action of the omasum or settle in the bottom of the reticulo-rumen (Hungate 1966). Although the two pools are closely mixed they behave differently, resulting in the simultaneous operation of different turnover rates. The quantity of digesta in the reticulo-rumen or the cumulative fill (Blaxter _et al_. 1956; Van Soest 1982), at any given time is the sum of residues left over from previous meals remaining in the reticulo-rumen. The interrelationships between the intake of food and water and the disappearance of digesta through digestion and passage result in a diurnal variation in cumulative fill.

The chemical composition of digesta depends on the diet, saliva, microbial activity and processes of absorption. Detailed information on composition is provided by Hungate (1966). Specific aspects of composition which may affect the quantity of digesta are considered where appropriate.

1.2.3 **Inputs to the fluid pool of digesta**

The volume of fluid in the reticulo-rumen represents about 15% of the total body water. In addition to water consumed, about 30% of the total body water flows into the reticulo-rumen as saliva in 24 hours (Engelhardt 1970). The reticulo-rumen serves as a water store (Hecker _et al_. 1964) in that water can be drawn from the reticulo-rumen to maintain cellular fluid levels during periods of water deprivation.

Reported fluid exchanges in the reticulo-rumen have been quite variable despite constancy of experimental conditions. The variation has been unpredictable both between and within animals (Hyden 1961; Warner & Stacy 1968b). The random fluctuations in flow rates and digesta volumes have not, however, prevented animals from having characteristically high or low values, or prevented animals from exhibiting characteristic responses to feeding or drinking (Warner & Stacy 1968b). Consistent differences between individuals in water intake have been observed in sheep (Warner & Stacy 1972a), but in general, water intake is proportional to dry matter intake (Grovum & Williams 1977; Ulyatt _et al_. 1984). Ingestion of food is the major factor which influences fluid movements into and out of the reticulo-rumen, due to its effects on saliva production and on the composition of digesta relative to blood.

Ingestion of food results in marked increases in rumen fluid volume, dilution rate and outflow to the omasum (Reid 1965; Stacy & Warner 1966; Warner & Stacy 1968b). Animals fed once daily show a characteristic pattern in the quantity of digesta in the reticulo-rumen, with the quantity maximal after feeding then slowly declining. Frequently fed sheep show no significant diurnal variation in digesta quantity (Minson 1966; Ulyatt _et al_. 1984). The volume of rumen fluid in grazing sheep exhibited a diurnal rhythm, varying by up to 15% from the 24 hour mean and with the lowest volume about midday (Corbett & Pickering 1983). The extent to which the volume of rumen fluid increases during feeding in animals fed once daily is influenced by the rate of food intake and the rate of outflow to the omasum. In some sheep the rate of outflow equalled the rate of inflow, with little change in volume (Warner & Stacy 1968b). Following the feeding period, the fluid dilution rate drops to levels similar to or less than those observed before feeding (Stacy & Warner 1966; Teeter & Owens 1983), although outflow rate from the reticulo-rumen remains elevated and digesta quantity decreases (Warner & Stacy 1968b). The changes in dilution rate associated with feeding have been attributed to saliva and to water entering across the rumen wall.

1.2.3.1 Saliva

The composition of saliva and the quantity of saliva produced may affect the composition and quantity of digesta in the reticulo-rumen. A brief outline of the classification of the salivary glands was given in Section 1.1.3. In addition to moistening food to assist chewing and swallowing, saliva supplies salts and water to the reticulo-rumen and buffers the acids produced during fermentation (Bartley 1976). Saliva is produced under nervous control (Kay 1958, 1966; Ash & Kay 1959; Kay & Phillipson 1959; Comline & Titchen 1957; Emmelin 1967).

Estimates of the total daily saliva secretion in cattle range from 33.5 to 190 ℓ (Bailey 1961a, Meyer _et al_. 1964; Putnam _et al_.

1966). Diet, level of intake, and the technique used to estimate total production contribute to this variation.

The composition of saliva depends on the site and rate of secretion and on the mineral status of the animal (Denton 1956; Phillipson & Mangan 1959; Bailey 1961b; Bailey & Balch 1961a; Kay 1960; 1966). The inorganic ion composition of parotid and mandibular secretions in sodium-replete cattle are shown in Table 1.1. Similar values occur in sheep (Kay 1960). Total mixed saliva resembles parotid saliva in its ionic composition (Kay 1966).

Table 1.1 Inorganic ion concentrations (mmol/ℓ) in bovine saliva.

	Na^+	K^+	Cl^-	HCO_3^-	HPO_4^{2-}
Parotid[a]	137-157	7-13.5	7.4-15.4	108-127	20-23
Mandibular[b]	13.6	13.9	30.9	15.7	0.5

[a] Phillipson & Mangan 1959, Bailey & Balch 1961a
[b] Phillipson & Mangan 1959

Salivary sodium is the most important source of sodium in the reticulo-rumen; the concentration in rumen fluid being proportional to, but lower than, that in mixed saliva (Bailey 1961b). Warner & Stacy (1972b) demonstrated with sheep that saliva was more important in maintaining osmolality and concentration of sodium in the reticulo-rumen following water loading than were movements of water or solute across the rumen wall. Dietary potassium is the major source of potassium in the reticulo-rumen, and the potassium concentration in rumen fluid is usually proportional to, but higher than, that in saliva (Bailey 1961b).

An inverse relationship exists between the concentrations of sodium and potassium in saliva, ensuring that the salivary osmotic pressure is maintained (Denton 1956). Sodium-replete animals have sodium:potassium ratios greater than about 18 whereas the ratio is less than 1 in animals deficient in sodium (Denton 1956, 1957; Towers 1983).

Feeding provides a potent stimulus for the secretion of both mandibular and parotid saliva. Mandibular secretion in sheep increases just before or at the start of feeding and remains elevated during the feeding period (Carr 1984). Parotid secretion peaks within 30-120 seconds of offering food, this peak being temporarily maintained before declining (Brightling _et al_. 1977; Carr & Titchen 1978; Patterson _et al_. 1982; Carr 1984). The decline was found to be more rapid if food was removed, occurring within 180 seconds of removal (Brightling _et al_. 1977; Patterson _et al_. 1982). As vigour of eating declines, and after feeding is complete, the rates of parotid and mixed secretion are often lower than those obtained immediately before feeding (Bailey & Balch 1961b; Wilson 1963; Putnam _et al_. 1966; Patterson _et al_. 1982; Carr & Titchen 1978; Carr 1984).

The peak in parotid secretion which occurs when food is offered has been accompanied by an increase in protein content (Brightling _et al_. 1977; Voigt _et al_. 1980; Patterson _et al_. 1982) and changes in the composition of the protein (Voigt _et al_. 1980). The duration of the increase in production of protein varied markedly between and within animals. The time taken to return to pre-feed levels ranged from 15 to more than 65 minutes in one sheep on two occasions (Patterson _et al_. 1982). Protein concentration also increased in mandibular saliva during feeding (Carr 1984).

The rates of production of both parotid and mixed saliva have been found to be at their lowest following feeding, gradually increasing to reach maximum rates just before the next meal (Bailey

& Balch 1961a,b; Wilson 1963). In sheep fed *ad libitum*, the mean secretion rates of parotid saliva during successive rest periods over 24 hours were found to be similar (Wilson 1963; Patterson *et al*. 1982) but large variation has been found between days and between sheep (Patterson *et al*. 1982).

Rumination temporarily increases parotid secretion (Denton 1957; Bailey & Balch 1961a), with an increase (Brightling *et al*. 1977) or little or no change (Patterson *et al*. 1982) in protein content. Rumen fluid on the tongue was found to stimulate secretion, but the act of chewing did not affect salivary secretion since sheep ground their teeth without affecting salivary output (Denton 1957; Somers 1957).

The decline in parotid secretion during feeding has been attributed to the inhibitory effects of continued eating (Bailey & Balch 1961b) and gastric distension (Wilson 1963), since high levels of ruminal distension inhibit parotid secretion (Comline & Titchen 1957; Phillipson & Reid 1958; Kay & Phillipson 1959). This does not explain results which have been obtained with sheep on different diets, where greater volumes of parotid saliva were produced on fresh grass than on hay despite an increased pasture intake of 5-6 times the mass on hay (Carr 1984). The decline has been attributed to a feedback effect from the increase in osmotic pressure of rumen fluid (Warner & Stacy 1977) and plasma (Warner & Stacy 1977; Carr & Titchen 1978) that occurs during feeding (Section 1.2.3.2). Addition to the reticulo-rumen of NaCl or hyperosmolal solutions of sodium and potassium salts (Bailey & Balch 1961b; Wilson & Tribe 1963; Warner & Stacy 1977) or butyric acid (Obara *et al*. 1972), and intravenous infusion of sodium and potassium salts, sucrose and mannitol (Beal *et al*. 1975a; Warner & Stacy 1977; Carr & Titchen 1978) have reduced the rate of parotid secretion. Sheep accustomed to drinking saline water showed lower parotid secretion rates than did those drinking rainwater (Tomas & Potter 1975). Intravenous infusions of hyposmolal solutions increased the parotid secretion rate in sheep (Warner & Stacy 1977; Carr & Titchen 1978). Part of the variation between animals in flow rate and composition of saliva may arise from variation in degree of hyperosmolality of reticulo-rumen contents during feeding, although this has yet to be demonstrated.

The salivary response to feeding and the total daily saliva production may be affected by the level of dry matter intake but there appears to be uncertainty over the response. The mean secretion rate of parotid saliva in sheep during rest (Wilson 1963), the total daily production of parotid saliva in sheep (Wilson & Tribe 1963) and the daily mixed saliva secretion in steers (Putnam _et_ _al_. 1966), were each found to increase as the dry matter intake increased. Contrary to this, Bailey & Balch (1961b) reported that the secretion rate of mixed saliva of cows at 2 and 8 hours after feeding decreased as intake increased. Putnam _et_ _al_. (1966) found that the mixed saliva production, expressed per kilogram of food eaten, decreased as intake increased but that the total daily production of saliva increased. Doyle _et_ _al_. (1982) found using sheep that the rate of parotid secretion increased during non-feeding periods (19.5 hours per day) with increasing intake, so that the quantity of saliva produced per 19.5 hours per 100 g dry matter was similar for all levels of intake. Part of the variation between experiments may have arisen from the method of collection. Collection from specific glands such as one parotid gland ignores contributions from the second parotid and from other glands. In addition, collections over short periods of time may have been extrapolated to 24 hours.

No clear relationship between the quantity of food consumed and the protein content of parotid saliva produced during feeding was found in sheep offered lucerne chaff (Patterson et al. 1982), indicating that factors other than the amount consumed affect the variation between animals in composition of saliva.

Sectioning of parotid nerves and ducts in sheep has resulted in reductions in dry matter intake, rate of passage of digesta and digestion of cellulose, and an increase in the dry matter content of digesta (Wilson 1964), indicating the importance of saliva to reticulo-rumen function. The extent to which variation between individuals in the quantity and composition of saliva modifies digestion and the passage of digesta out of the reticulo-rumen is not known.

1.2.3.2 **Net fluid flux across the reticulo-rumen wall**

Movements of water across the reticulo-rumen wall may influence both the composition and the quantity of digesta in the reticulo-rumen. Movements both into and out of the reticulo-rumen are probably influenced by osmotic gradients between the rumen fluid and plasma (Dobson 1984), but opinions on the direction of net flux vary. Osmotic gradients arise from the ingestion of food, which results in rapid increases in rumen fluid osmolality, contents of dry matter and ash, and concentrations of potassium, total nitrogen, ammonia and volatile fatty acids, causing digesta to become hypertonic to blood (Warner & Stacy 1965, Ternouth 1967, Bergen 1972, Dooley & Williams 1975). Rumen fluid osmolality before and after feeding may differ between animals (Warner & Stacy 1965) but peak osmolality does not appear to be related to the amount of food eaten (Dooley & Williams 1975). No evidence was found that drinking was regulated in order to maintain the osmolality of rumen fluid within specific limits (Warner & Stacy 1965).

Ternouth (1967) reported a high net influx of water into the reticulo-rumen in sheep, even when reticulo-rumen digesta was hypotonic to blood, and concluded that this inflow decreased the osmotic pressure of the digesta. Warner & Stacy (1968a,b) criticised this work on technical inadequacies and the mathematics employed. It is probable that the lowering of osmotic pressure occurs by solute absorption from the reticulo-rumen rather than by fluid dilution (Stacy & Warner 1966; Warner & Stacy 1972b; Dobson et al. 1976). The increase in rumen fluid osmolality and electrolytes as a result of feeding has been associated with an increase in plasma osmolality (Warner & Stacy 1965; Ternouth 1967; Carr & Titchen 1978). Carr & Titchen (1978) offered sheep chaffed lucerne hay once daily and observed increases of 2-4 mosmol/kg within 5 minutes of the start of feeding, and 12-30 mosmol/kg over the subsequent 3 hours. Whereas rumen fluid osmolality may remain elevated for 3-4 hours after feeding before declining (Warner & Stacy 1965; Bergen 1972), plasma values have remained elevated for up to 10 hours (Warner & Stacy 1965; Carr & Titchen 1978). Sheep fed hourly show less marked rumen fluid and plasma osmolality responses than do those fed once daily (Dooley & Williams 1975).

In contrast to the results of Ternouth (1967), Stacy & Warner (1966) found that there was no appreciable net influx of water across the rumen wall of sheep before, during, or after feeding. All of the net inflow was accounted for by saliva. Similarly, Warner & Stacy (1968b) and Willes et al. (1970) concluded that little water moved into the reticulo-rumen across the rumen wall during feeding, despite hyperosmolality (400-500 mosmol/kg) of reticulo-rumen contents relative to blood (Warner & Stacy 1968b). Water was absorbed from the reticulo-rumen into the blood from isotonic or hypotonic contents, but the rates were low (Warner & Stacy 1968b). In contrast to this again, Parthasarathy & Phillipson (1953) found that water moved out of hypotonic and into hypertonic reticulo-rumen contents. Engelhardt (1970) concluded that there was no net flux across the wall when the osmolality of the contents was in the range of 265 to 320 mosmol/kg, and that a sigmoidal relationship existed between rumen content osmolality and net flux outside this range. However, Dobson et al. (1970, 1976) using cows with an isolated ventral sac, and Warner & Stacy (1972b) using sheep, both found this relationship to be approximately linear. Water moved into hyperosmolal contents and out of hyposmolal contents, with the minimum net flux occurring at about 330-335 mosmol/kg. Dobson et al. (1970) found that this value, of about 330 mosmol/kg, depended on the presence of CO_2 in the gas phase since water movements occurred against an osmotic gradient in the presence of CO_2, but Stacy & Warner (1972b) found this effect of CO_2 to be relatively minor.

Dobson et al. (1976) and Dobson (1984) concluded that both the blood flow and the hydraulic conductance of the epithelium influenced the absorption of solutes. Epithelial conductance became more important at limiting water movements at high blood flows stimulated by osmotic gradients (Dobson et al. 1976). Movement of water in the absence of an osmotic gradient could occur by the coupling of water and solute fluxes but Dobson et al. (1976) concluded that this had a minor role. General conclusions appear to be that the most important route for removal of water from the reticulo-rumen of sheep and cattle is by passage to the omasum, and that net flux across a healthy epithelium is not large and plays only a minor part in fluid exchange (Engelhardt 1970; Warner & Stacy 1972b). The extent or possible causes of variation between animals in net water flux is not known.

1.2.3.3 **Circulatory changes in response to feeding**

The movements of water and minerals from the circulation into the reticulo-rumen, particularly during feeding, are associated with major changes in the circulation and renal function (Scott 1975).

Transfer of bicarbonate ions into saliva was found to be associated with a decrease in the basicity of plasma in the jugular vein, to which the kidneys responded by lowering the pH of the urine (Billitzer & Jarrett 1970). The volume of plasma and extracellular fluid decreased and the heart rate increased in sheep within 15 minutes of being offered dry feed (Blair-West & Brook 1969; Christopherson & Webster 1972). The length of time that plasma volume remains at a minimum may depend on the level of intake (Dooley & Williams 1975). Renal conservation of sodium and water occurred within 30 minutes of feeding, continuing for 2-3 hours (Blair-West & Brook 1969). The effect of feeding on the vascular volume and sodium content and, therefore, on circulatory and renal regulatory systems, depends upon the rate at which extravascular fluid enters the system. Renin was released into the circulation of sheep which ate their food rapidly, but slow eaters did not show this response (Blair-West & Brook 1969). This activation of the renin-angiotensin system was considered to be a direct consequence of the fall in circulatory volume. Stacy & Brook (1965) observed a post-prandial retention of water and attributed it to an antidiuretic substance, assumed to be antidiuretic hormone (ADH), which was released in response to the increase in plasma osmotic pressure. However, Blair-West & Brook (1969) found that the onset of antidiuresis coincided with the time of rapid decrease in circulatory volume, therefore release of ADH was a result of the increased circulating angiotensin, in response to the decreased volume (Scott 1975).

1.2.3.4 **Effect of electrolyte intake on fluid movements**

Intakes of sodium and potassium and their movements into and out of the reticulo-rumen have been shown to influence the kidney and other body mechanisms involved in maintaining fluid and electrolyte balances (Scott 1975). These balances may affect both the flow of fluid into and out of the reticulo-rumen and the quantity of digesta in the reticulo-rumen.

There is an inverse relationship between the concentrations of sodium and potassium in rumen fluid (Warner & Stacy 1965). In sheep and cows on roughage diets, resting values of 120-170 mmol/ℓ of sodium and 10-30 mmol/ℓ of potassium have been reported (Bailey 1961b; Warner & Stacy 1965, 1972a; Stacy & Warner 1966). Under high dietary potassium and/or when sodium deficient, values for sodium can decrease to 40-80 mmol/ℓ and those for potassium increase to 60-80 mmol/ℓ (Bailey 1961b; Warner & Stacy 1965, 1972a; Dobson _et al_. 1966; Scott 1967).

Potassium has been shown to move passively across the rumen epithelium in response to its concentration in the rumen and the gradient between rumen and blood (Parthasarathy & Phillipson 1953; Scott 1967; Warner & Stacy 1972b). Absorption of sodium depends on the concentration of potassium and the osmotic pressure as well as the sodium concentration (Stacy & Warner 1966; Warner & Stacy 1972b). Dobson (1959) and Warner & Stacy (1972b) found that sodium was transported against concentration and electrochemical gradients so that it was removed from the resting reticulo-rumen at a greater rate than was water. This suggested that the absorption of sodium from the reticulo-rumen was the major mechanism maintaining the resting contents hypotonic to blood.

As well as exerting effects on sodium in the reticulo-rumen, potassium acts on the kidney to alter sodium excretion. High potassium diets or the addition of potassium salts to the rumen has caused marked increases in urinary potassium and sodium excretion (Dobson _et al_. 1966; Dewhurst _et al_. 1968; Simpson 1969; Warner & Stacy 1972a). These effects of the intakes of sodium and potassium on body fluid and electrolyte metabolism were demonstrated in sheep by Dobson _et al_. (1966) by changing diets, and by Warner & Stacy (1972a) by varying the mineral content of one diet. These workers found that the combination of high potassium and low sodium intakes resulted in increased water consumption and urine production, an increased sodium excretion in the urine, and an increased concentration of potassium and decreased concentration of sodium in rumen fluid and saliva. Shifting back to a low intake of potassium reversed all of the effects. Increasing the potassium intake when the sodium intake was high affected the concentrations of sodium and potassium in the rumen fluid, as for the low sodium intake, but did not necessarily change the sodium:potassium ratio in the saliva. A low sodium intake, without an increase in potassium intake, had no effect on urinary sodium excretion, and only a slow decrease in the concentration of sodium and increase in that of potassium in rumen fluid and saliva were observed. If sodium intake was increased to very high levels no marked changes were observed in sodium and potassium concentrations in rumen fluid and saliva, although Warner & Stacy (1972a) found that both the rumen fluid volume and flow rate were increased by about 15%. Nearly all of the extra sodium consumed was excreted in the urine. Thus the potassium intake was responsible for the major changes observed in the salivary sodium:potassium ratio and in the sodium excretion in urine. Dobson et al. (1966) observed an increase in live weight, and tentatively ascribed it to a change in the volume of rumen fluid caused by retention of sodium in the rumen, when shifting from a high potassium (grass) to a low potassium (hay and meal) diet. Warner & Stacy (1972a) did not obtain this response, but their rumen fluid volume and flow rate data appeared to be more variable than other parameters recorded. They observed that the time required to reach a new equilibrium was much longer after lowering the potassium intake than when it was raised.

Similar effects of the dietary sodium and potassium intake on the sodium:potassium ratio in mixed saliva of sheep and cows were reported by Dobson (1963) and Dobson & McDonald (1963). There was marked variation between animals in the timing and extent of the responses. Sodium and potassium may also differ in their effects on salivary flow rate. Intravenous infusion of KCl has been shown to decrease the rate of flow of parotid saliva in intact and adrenalectomised sheep, whether sodium replete or deplete (Beal et al. 1975a,b). The secretion rate of parotid saliva was unaffected (Beal et al. 1975a) or decreased (Carr & Titchen 1978) by the intravenous infusions of hyperosmolal NaCl solutions. Infusions of hyperosmolal solutions have also decreased the rate of reticular contraction in sheep (Carr & Titchen 1978).

The observed decrease in salivary sodium:potassium ratio in sodium deficient animals is believed to be due to an increased secretion of aldosterone by the adrenal gland. The evidence for this was reviewed by Blair-West et al. (1967).

Electrolyte intake has been shown to affect the dilution rate, volume of rumen fluid and outflow from the reticulo-rumen. The infusion of artificial saliva into the rumen (Harrison _et al._ 1975, 1976), or the inclusion in the diet of artificial saliva (Thomson _et al._ 1978), NaCl or $NaHCO_3$ (Hemsley _et al._ 1975; Rogers _et al._ 1979, 1982; Rogers & Davis 1982a,b) were found to increase the rumen fluid dilution rate. Thomson _et al._ (1978) found that the effect of salts on dilution rate occurred for animals fed at frequent intervals but not for those fed twice daily. In contrast to these results, Chamberlain & Thomas (1980) observed a reduction in dilution rate after the addition of 4 ℓ of artificial saliva to the rumen, compared to the infusion of 2 ℓ of water per day. This result was obtained with sheep offered a concentrate diet which was known to be associated with low rates of production of bacterial protein in the reticulo-rumen, thus diet may be important in determining the extent of the response. Hadjipanayiotou _et al._ (1982) found that the level of inclusion in the diet of artificial saliva salts influenced the effect on dilution rate; the addition of 40 g per day of salts to a concentrate and dried grass diet increased dilution rate, but 20 g had no effect. Sheep with low dilution rates have shown the greatest responses to salt treatments (Harrison _et al._ 1975), therefore the results may differ between experiments due to the animals, particularly where few animals are used. The effect of salts on the dilution rate is due, at least in part, to an increase in water intake (Hemsley 1967; Hemsley _et al._ 1975; Rogers _et al._ 1979, 1982; Rogers & Davis 1982a,b), although infusion of water alone has no effect on dilution rate (Harrison _et al._ 1975). The intraruminal infusion of NaCl had no effect on the rumen fluid dilution rate and outflow when access to water was denied (Bergen 1972). Hemsley _et al._ (1975) found that sheep supplemented with NaCl increased their water intake by 2 ℓ per day but that outflow from the reticulo-rumen was increased by 5-6 kg/day, and the mean residence time of soluble marker decreased from 20 to 12 hours. The volume of rumen fluid was not changed. Water was absorbed from the reticulo-rumen on both diets but less was absorbed on the salt supplemented diet than on the control diet, thus a decreased net absorption contributed to the increased outflow. Offering saline drinking water to sheep has resulted in increases in fluid intake, rumen fluid osmotic pressure and fluid outflow from the reticulo-rumen on roughage rations (Potter et al. 1972), although Tomas & Potter (1975) were unable to demonstrate this effect on outflow. The volume of rumen fluid in sheep was increased after feeding, but not before, by the addition of NaCl to a high protein diet (Hemsley 1975). The effect of salts on rumen fluid volume may be affected by diet as Rogers et al. (1979) found that the volume of rumen fluid was decreased on a concentrate diet and increased on a roughage diet by adding salts to the rumen.

The increases in dilution rate effected by salts have been associated with changes in the proportions of volatile fatty acids in the rumen (see Section 1.2.5.4).

Other interactions may occur between inorganic ions in the ruminant digestive tract. For example, the dietary potassium intake and potassium:sodium ratio have been shown to affect the net absorption of magnesium from the reticulo-rumen (Greene et al. 1983; Care et al. 1984). Pasture species appear to differ in their effects on movements of minerals across the wall of the gut in the ruminants. Grace et al. (1974) found that both the net secretion of sodium into the stomach region (rumen, reticulum, omasum and abomasum) and the net absorption of sodium from the large intestine in sheep offered perennial ryegrass (Lolium perenne L.) were twice those than when the diet was white clover. In sheep offered clover, similar quantities of potassium were absorbed from the stomach region and small intestine, whereas with grass the net absorption of potassium from the small intestine was about three times that occurring from the stomach region. The importance of the stomach region in potassium absorption on the clover diets may have been because the potassium content of clover was at least 30% higher than that of the grass (Grace et al. 1974), and absorption of potassium from the reticulo-rumen depends on its concentration in digesta (Warner & Stacy 1972b).

Many herbage diets in New Zealand are high in potassium and low in sodium relative to the requirements of ruminants (Smith & Middleton 1978), and lactating cows have been found to be in marginal Na status on some pastures (Towers 1983). There is a lack of information on the effects of mineral supplements or mineral content of the diet on the rumen fluid volume and dilution rate in ruminants, particularly cattle, consuming fresh herbages.

1.2.4 Inputs to the solid pool of digesta

1.2.4.1 Intake and the capacity of the reticulo-rumen

The quantity of food consumed, relative to body size, may affect the quantity of digesta in the reticulo-rumen (Section 1.2.4.2) and has a marked affect on digesta turnover (Section 1.2.5.3).

The control of food intake has been extensively reviewed (Balch & Campling 1962; Arnold 1970; Baumgardt 1970; Campling 1970; Forbes 1970; Bines 1971; Baile & Forbes 1974). It is a complex process, involving interrelationships between the hypothalamus and metabolites, hormones and receptors, operating for short term (meal size and frequency) and long term (energy balance) control.

Arnold (1970) reviewed factors affecting the selection and ingestion of food, including sensory appraisal, palatability and behavioural factors. Control of intake via factors such as volatile fatty acids and other organic acids, pH and osmolality of reticulo-rumen contents, amino acids, metabolites and glucostatic mechanisms has been reviewed in detail by Baile & Forbes (1974), and is not discussed in this review.

Gastrointestinal fill has been the basis for many hypotheses and experiments on the control of food intake (Blaxter _et_ _al_. 1961; Campling & Balch 1961; Campling _et_ _al_. 1961; Freer & Campling 1963; Ulyatt _et_ _al_. 1967). It is not always clear whether apparent relationships between characteristics of fill and food intake are cause and effect or whether they result from other food qualities (Baile & Forbes 1974).

Blaxter _et_ _al_. (1961) offered hays of differing quality to sheep and found that the quantities of digesta in the reticulo-rumen were similar at the end of the meal. They suggested that ruminants eat to a constant distension and that this is determined by the digestibility of the diet and the rate of passage of undigested residues. Johns _et_ _al_. (1963) suggested that this may be an oversimplification, as they obtained an effect of diet on rumen size relative to body weight and on rumen epithelium development. Physical factors which regulate intake appear to become less important as the diets become increasingly digestible (Conrad _et_ _al_. 1964; Hutton _et_ _al_. 1964; Bines & Davey 1970). Egan (1970) found that both intake and the quantity of digesta in the reticulo-rumen after feeding were increased by infusions of casein into the rumen or duodenum, suggesting that the protein status of the animal may affect gastrointestinal fill after feeding.

The higher voluntary intake of legume species relative to that of grasses is associated with a lower retention time in and an increased flow of fluid from the reticulo-rumen (Ulyatt 1969; Thornton & Minson

1973; Moseley & Jones 1979) and a decreased weight of reticulo-rumen contents (Johns _et al_. 1963; Ulyatt 1969). At similar dry matter intakes, the flow of digesta from the reticulo-rumen on white clover was higher than that on perennial ryegrass but not that on short-rotation ryegrass (_Lolium multiflorum_ x _perenne_) (Ulyatt & MacRae 1974). Sheep offered grass had higher weights of digesta but similar weights of dry matter in the reticulo-rumen than did sheep offered clover (Moseley & Jones 1984).

Intake is usually expressed relative to body size (absolute or metabolic) although intake relative to reticulo-rumen size is the factor which affects the quantity and turnover of digesta. Reticulo-rumen capacity, or size, has been estimated for ruminants but the value obtained depends on the method used (Tulloh & Hughes 1965). Alternatively, estimates of the weight of digesta in the reticulo-rumen at maximum fill have been made, but these estimates are affected by technical problems (Van Soest 1982), and are usually related to specific proportions of the digesta if markers are used (Nutt _et al_. 1980).

Positive relationships exist for a given diet between live weight and the empty rumen or reticulo-rumen weight or water-filled capacity in sheep (Johns _et al_. 1963; Purser & Moir 1966) and between structural size of the animal and reticulo-rumen fill on a bulky diet in cattle (Nutt _et al_. 1980). However, when comparing perennial and short-rotation ryegrass and white clover diets, Johns _et al_. (1963) found that the diet which resulted in the lowest live weights also resulted in the largest rumen size. Structurally large cows did not have a larger reticulo-rumen capacity when capacity was expressed in proportion to live weight (Nutt _et al_. 1980).

Table 1.2 Reported correlations between intake and the quantity of fluid and solid digesta in the reticulo-rumen.

Species	Frequency of feeding (hours)	Diet	Hours after feeding[a]	Correlation[b]		Method[c]	Source
				F	S		
sheep	1	chaffed lucerne hay		+	+	M	Grovum & Williams (1977)
sheep	1	chaffed lucerne hay		+	+	M	Ulyatt *et al.* (1984)
sheep	1	chaffed pasture hays		+	+	R	Minson (1966)
sheep	1	chopped oaten hay		0	+	M	Egan & Doyle (1985)
sheep	4	lucerne pellets	2	0	+	M	Mudgal *et al.* (1982)
sheep	24	chaffed lucerne hay	2,14 & 24	+	+	Sl	Ulyatt *et al.* (1984)
sheep	24	clover & grass hays	0	+	+)	R	Moseley & Jones (1984)
			24	0	0)		
lambs	24	pelleted hay & concentrates	24	-	-	M	Margan *et al.* (1982)
sheep	24	hay & supplements	24	0	+	R	Pearce (1967)
steers	24	lucerne hay	0	-		M	Adams & Kartchner (1984)
cows	24	clover-grass hay	0 & 24	+	+	R	Campling *et al.* (1961)

[a] Time of estimate relative to feeding for feeding intervals greater than 1 hour
[b] F, fluid; S, solid; +, positive correlation; 0, no correlation; -, negative correlation
[c] Method used to obtain estimate, M, marker; Sl, slaughter; R, digesta removal through cannula

Capacity has been shown to be affected by physiological state, including fatness, pregnancy, and lactation. Cows offered feed _ad libitum_ consumed 24% more when they were thin, than when they were fat. This was attributed to the feedback of metabolites on energy status (Bines & Morant 1983). Nutt _et al_. (1980) found that fatter, heavier cows had smaller volumes of digesta in the reticulo-rumen when expressed as a percent of body weight, than did thinner, lighter cows, and suggested that reticulo-rumen capacity was proportional to the fat-free body mass. It has also been suggested that internal fat deposits physically limit reticulo-rumen capacity (Tayler 1959). Data reviewed by Forbes (1970, 1971) suggested that pregnancy may decrease capacity through physical compression by the uterus. Weston _et al_. (1983) found that pregnancy decreased the digesta load in the reticulo-rumen and increased the clearance rate of water soluble markers in cattle offered herbage. In cows the effects of pregnancy may be confused by effects of lactation. Lactating cows were found to have 32-40% larger reticulo-rumen size than their dry twin mates (Tulloh & Hughes 1965).

1.2.4.2 Effect of level of intake on the quantity of digesta in the reticulo-rumen

An increase in intake may be associated with an increase in the quantity of digesta in the reticulo-rumen, an increase in passage rate, or both.

Animals fed at frequent intervals have a relatively constant quantity of digesta in the reticulo-rumen compared to those fed once daily (Ulyatt _et al_. 1984). Table 1.2 summarises some reported correlations between the level of intake and the quantity of fluid and solid digesta in the reticulo-rumen. Variable results have been obtained, but in general positive correlations have been reported, particularly when the estimates of the quantity of digesta were made directly by weighing the contents. Of the results not showing positive correlations, Adams & Kartchner (1984) (Table 1.2) used a marker technique which has presented problems with interpretation of results (Warner & Stacy 1968a) and sheep used by Pearce (1967) were given food through the fistula. The summarised results suggest that the level of feeding is a major factor affecting the quantity of digesta in the reticulo-rumen, but that other factors such as diet and feeding regime may be important. Marked variation between animals can occur in the intake-digesta quantity relationship (Campling *et al.* 1961; Pearce 1967). Evident from Table 1.2 is the predominance of the use of dried roughage diets, and the lack of use of fresh herbage diets for studies involving digesta measurements.

1.2.5 Passage of digesta from the reticulo-rumen

Outflow of digesta from the reticulo-rumen occurs simultaneously with digestion and factors which influence passage rate affect the length of time feed residues are available to the micro-organisms. Factors affecting rate of digestion in the reticulo-rumen have been discussed by Mertens (1977), Akin (1982) and Van Soest (1982). Products of microbial digestion are eructated, absorbed through the rumen wall or carried with fluid and undigested residues out of the reticulo-rumen. The relationships between intake, ration composition, digesta turnover, and digestion are complex. Some of the dietary factors that affect digesta turnover include protein, carbohydrate and lignin content (Mertens 1977; Bull *et al.* 1979), the proportion of roughage in the ration (Evans 1981a,b; Owens & Isaacson 1977), and grinding of the diet (Blaxter *et al.* 1956; Weston & Hogan 1967).

Discussion of these factors is outside the scope of this review. Consideration is given to the relationships between passage rate and reticulo-rumen motility, digesta particle size, and intake.

1.2.5.1 **Motility**

Passage out of the reticulo-rumen through the reticulo-omasal orifice can only occur when the orifice is open and the pressure in the reticulum exceeds that in the omasal canal. This has been shown to occur during the second phase of the reticular contraction (Stevens et al. 1960; Bost 1970; McBride et al. 1983). The contraction cycles of the reticulum and rumen were briefly outlined in Section 1.1.7.

Eating stimulates an increase in motility of the reticulum and rumen. An increased frequency of contraction sequences, ratio of B to A sequences, and rumen contraction strength has been observed in sheep during eating (Reid 1963). The extent and persistence of the response depended on the animal species, dietary factors including the amount and nature of food consumed, frequency of feeding, and the amount and nature of food consumed in previous meals (Reid 1963; Colvin et al. 1978; Waghorn & Reid 1977, 1983). The level of feeding and the nature of the diet were reflected in times spent eating, ruminating and resting, which have different contraction frequencies (Waghorn & Reid 1977).

Ash & Kay (1959) demonstrated with sheep that tactile stimulation of the oesophagus, cardia, reticulo-ruminal fold, reticulo-omasal orifice and walls of the reticulum and anterior pillar were effective in increasing the frequency of contraction of the reticulum, but that distension of the rumen with a balloon inhibited motility. Stevens & Sellers (1959) found that a graded increase in pressure in the reticulum and rumen of cows increased the rate of both A and B sequences and of eructation, but characteristics of the rumen contents also affected the frequency and amplitude of contraction. Removal of contents decreased the rates of contraction, and replacement of contents with fluid inhibited the rate of contraction of the B sequence. They concluded that distension may provide a major stimulus for the reflex control of A sequences but that the frequency of B sequences depended on other stimuli, perhaps tactile.

Passage through the omasum is controlled by conditions within the reticulum, rumen and abomasum (Sellers & Stevens 1966). The reticulo-omasal orifice and omasal leaves are thought to act as a filter to prevent the passage of large digesta particles, and backflow of digesta, presumably to clear the omasum of large particles, has been observed (Stevens _et al_. 1960). Although dimensions of the orifice are greater than the mean size of particles found in the omasum (McBride _et al_. 1983) it is thought that particle breakdown is of major importance in the determination of outflow.

Data obtained by Freer _et al_. (1962) and Freer & Campling (1965) with cows and by Ulyatt _et al_. (1984) with sheep suggested that regulation of outflow in response to changes in intake occurred by a change in the amount of digesta passed out of the reticulo-rumen per A sequence contraction rather than by changing the frequency of contraction. It is not clear whether, or to what extent, passage occurs during B sequences (Ulyatt _et al_. 1984).

In addition to affecting the outflow of digesta, motility of the reticulo-rumen is considered to be particularly important in promoting absorption across the rumen wall by the mixing of contents and the presentation of solutes and fermentation products to the epithelium (Dobson 1984), suggesting that an "efficiency" factor associated with reticulo-rumen movements may affect both absorption and passage.

1.2.5.2 **Particle size**

Many workers have demonstrated, using both sheep and cattle and a range of diets and conditions, that particulate matter is retained longer in the reticulo-rumen than is fluid (Grovum & Williams 1973, 1977; Faichney 1975; Faichney & Griffiths 1978; Lemerle 1981; Haaland & Tyrrell 1982; Okeke et al. 1983; Egan & Doyle 1984; Ulyatt et al. 1984). Retention of particulate matter was also found to be longer than that of fluid in the abomasum, but retention times of particulate and fluid markers in the hindgut were similar (Grovum & Williams 1973; Faichney 1975). However, although it is convenient to label phases as fluid and particulate, these labels do not represent discrete phases and a range in flow rates with particle size exists. Egan & Doyle (1984) found with sheep continuously offered chopped oaten hay that apparent fractional outflow rates of particles increased with decreasing length (7 size categories ranged from >2.0 mm to <0.15 mm), although all particles less than 0.5 mm in length had similar outflow rates. The outflow rate of ^{103}Ru-P (^{103}Ru-labelled tris-(1,10 phenanthroline)-ruthenium (II) chloride), commonly used as a particulate marker, was similar to rates obtained for particles of 0.5 mm length or less, but was higher than the rates estimated for larger particles or for total dry matter.

The threshold size of particle for escape from the reticulo-rumen has been estimated for sheep as 1 mm in length (Ulyatt et al. 1976; Reid et al. 1977) and for both sheep and cattle offered tropical forages at 1.18 mm in length (Poppi et al. 1980, 1985), although Ulyatt et al. (1985) suggested that the size for cattle was 1.5 to 2 times that for sheep. The different estimates have arisen from the use of arbitrarily selected sieve sizes; a proportion of larger particles will always leave the reticulo-rumen. The estimated threshold size appears to be unaffected by dietary and physiological factors such as intake, digestibility, physical form of the diet and body size (Ulyatt et al. 1985). A threshold size alone does not determine escape, since a large proportion of particles in the reticulo-rumen are less than 1 mm (Pearce 1967; Reid et al. 1977; Poppi et al. 1981b). Troelsen & Campbell (1968) and Welch (1982) suggested that three-dimensional characteristics may be more important than particle size (length), and Campling & Freer (1962) and Evans et al. (1973) found that particle density was important.

Location of particles in the reticulo-rumen has been shown to affect their transit time to the reticulo-omasal orifice, with faster times occurring from ventral than from dorsal sites (Welch 1982). The greatest proportion of coarse particles occurs in the dorsal sac (Evans et al. 1973). McBride et al. (1984) observed, using endoscopy, that large particles (10 mm length) could pass through the reticulo-omasal orifice of cattle, and suggested that they do not normally do so because they are not of the proper size or density to occupy a ventral location in the cranial sac of the rumen.

Particle size is decreased by chewing during feeding and rumination and by microbial action. The extent of physical disruption of roughages depends on characteristics of the plant and on variation between animals in the extent and efficiency of breakdown. The contribution of chewing during feeding has been reviewed by Ulyatt et al. (1985). The extreme physical disruption which occurs during eating both decreases the size of particles and enables increased accessibility of microbial enzymes (Pond et al. 1984). Reid et al. (1979) using sheep found that the initial chewing of lucerne hay reduced the proportion of particles greater than 1 mm in length by 50% or more. Lee & Pearce (1984) obtained decreases of 30-40% in the proportion of particles greater than 1 mm in length when feeding 5 chaffed roughages to steers, with a significant difference between animals in the effectiveness of chewing. Chewing reduced the proportion of particles greater than 1.18 mm in length to a greater extent in leaf than in stem portions of grasses (Poppi et al. 1981b).

Rumination has been found to start when the concentration in the reticulo-rumen of particles of low density was at a maximum, and that of high density was at a minimum (Evans et al. 1973). The time spent eating and ruminating per unit of roughage intake was the same at all intake levels (Freer et al. 1962; Freer & Campling 1965). Depressing rumination by fitting muzzles increased the retention time of digesta (Pearce & Moir 1964).

Reduction of particle size by microbial action is thought to have a minor contribution to the total reduction of size necessary for passage out of the reticulo-rumen, but may be important for reducing the particle size of some feedstuffs (Ehle et al. 1982; Murphy & Nicoletti 1984).

There are differences between pasture species in characteristics of particle size, and these are likely to be related to differences in passage rate out of the reticulo-rumen. Legumes have a more compact, "blockish" particle structure compared to thread-like grass particles (Troelsen & Campbell 1968; Moseley & Jones 1984). The rate of particle size breakdown in the reticulo-rumen was found to be more rapid for clover than for grass (Moseley & Jones 1984). The proportion of large particles in digesta increased with increasing forage maturity (Troelsen & Campbell 1968), although Poppi et al. (1985) found no effect of maturity of forage on the resistance of each particle size to leave the reticulo-rumen.

Contractions of the reticulo-rumen are not thought to be of importance to the breakdown of particles (Ulyatt et al. 1985), therefore chewing during eating and rumination is the major factor reducing particle size. Characteristics of the diet and differences between animals affect the effectiveness of chewing. However, while the major rate-limiting step to clearing residues from the reticulo-rumen is passage through the reticulo-omasal orifice, it is not clear whether the rate of particle size reduction influences this mechanism (Ulyatt et al. 1985).

1.2.5.3 **Effect of intake on digesta turnover**

An increase in dry matter intake is associated with an increase in the rate of passage of both fluid and solid digesta out of the reticulo-rumen, and is often associated with a decrease in the retention time of digesta (Minson 1966; Thornton & Minson 1972, 1973; Grovum & Williams 1977; Mudgal et al. 1982).

Evans (1981a,b) concluded from reviewing the literature that the level of intake, on either a body weight or metabolic body weight basis, was a major factor affecting both liquid and solid turnover rates in the reticulo-rumen of both sheep and cattle, but that the relationship between intake and turnover was relatively poorer for solid than for liquid. Ulyatt et al. (1984), however, using sheep offered chopped lucerne hay, found that although increasing the intake increased the rate of passage of cellulose, hemicellulose and fibre out of the reticulo-rumen, retention times and fractional outflow rates were not altered because of an increase in the volume of digesta. Their range of intake, from 1 to 1.5 times maintenance, was relatively small compared to other reported trials.

An increase in water consumption associated with a change in diet has been associated with an increased outflow of rumen fluid from the reticulo-rumen (Rumsey et al. 1979), similar to that observed when water intake increases in response to mineral supplementation of the diet.

1.2.5.4 Effect on micro-organisms of dilution rate and rate of passage of digesta

The distinction between fluid and particulate pools does not apply to the microbial population in the reticulo-rumen. Microbes exist in free suspension, are loosely attached to or temporarily trapped in solid digesta, or are attached to solid digesta. Microbial populations show a characteristic growth curve dependent on residence time and generation interval, and as the dilution rate is altered, populations may change in type or metabolism (Hungate 1966; Owens & Isaacson 1977). Protozoa have been found to be sequestered in the reticulo-rumen (Weller & Pilgrim 1974). High dilution rates favour organisms having short generation times and the efficiency of production of microbial protein has been found to be greater at high dilution rates (Kennedy et al. 1976; Owens & Isaacson 1977). Increasing the dilution rate by the inclusion of sodium and potassium salts in the diet has improved the efficiency of microbial protein synthesis (Harrison et al. 1975, 1976). There was an inverse relationship between the molar proportion of propionate and the dilution rate when the dilution rate was changed by inclusion of salts (Harrison et al. 1975, 1976) or by a change in diet (Hodgson & Thomas 1975), although the dilution rate and the efficiency of microbial synthesis can be increased by exposure of sheep to cold temperatures without changing the pattern of fermentation (Kennedy et al. 1976). High dilution rates have been associated with bacterial, but not protozoal, population changes (Latham & Sharpe 1975; Thomson et al. 1978), or with a decrease in microbial population but not necessarily its metabolic activity (Potter et al. 1972). Hemsley et al. (1975) found that higher dilution rates were associated with a decrease in fermentation of organic matter and a reduced protozoal population in the reticulo-rumen. An increase in the volume of digesta and reduction in the dilution rate after injection into sheep of atropine sulphate was associated with an increase in the numbers of protozoa and bacteria (Thomas & Hodgson 1979). In contrast, when offered a concentrate diet sheep with low quantities of dry matter and water in the reticulo-rumen and low digesta retention times, had a large protozoal population compared to sheep with a high quantity of digesta (Faichney & Griffiths 1978). Changes in microbial population in response to changes in dilution rate or retention time of digesta appear, therefore, to be characteristics of the animal and the diet.

1.2.6 Evidence for a genetic basis for the quantity of digesta in the reticulo-rumen

The existence of differences between breeds in the quantity of digesta and rate of digesta turnover may suggest a genetic basis for the differences observed. Differences between breeds in digesta quantities, retention times and turnover rates have been reported for sheep (Margan et al. 1982; Weston & Cantle 1983) and cattle (Kennedy 1982) though in the latter the comparison was confounded by a difference in intake relative to live weight.

Considerable variation between animals in quantity, composition and turnover rate of digesta in the reticulo-rumen and in fluid flows through the reticulo-rumen have been reported. Warner & Stacy (1965) found that the fasting levels of the osmolality, dry matter content, and concentrations of Na and K in rumen fluid were characteristic of individual sheep. Responses to factors which tend to increase osmolality might also, therefore, be expected to vary from sheep to sheep (Faichney et al. 1980-81). Warner & Stacy (1965) found marked variation between sheep in responses to feeding in osmolality of rumen fluid. They also noted (Warner & Stacy 1968b) that two sheep could have similar body weights and rumen fluid dilution rates but distinctly different volumes of rumen fluid and net inflow rates of fluid to the reticulo-rumen. There was no correlation between rumen fluid volume and net inflow rate. Purser & Moir (1966) attributed larger resting volumes of digesta to sheep which were rapid eaters. Warner & Stacy (1968b) also found this tendency but the relationship was not significant.

When comparing digesta turnover and reticulo-ruminal fill of cows during the dry period and at three stages of lactation, Hartnell & Satter (1979) found twofold differences in turnover rate between individual cows. These differences were larger than those due to the change in physiological state. There were large differences in visual appearance and size of reticulo-rumen fill. For example, two cows with the same water intake had a 30 kg difference in weight of rumen fluid.

Faichney & White (1977) found that marked differences existed between sheep in the mean retention time of soluble marker in the reticulo-rumen and in the flow of water from the reticulo-rumen. The proportion of variance accounted for by the differences between sheep in these parameters was, respectively, 38% and 73%.

Differences between animals in fluid flows through the reticulo-rumen have not been explained by differences in intake (Egan et al. 1975) or by patterns of fermentation (Thomas & Hodgson 1979). Variation between individuals in the mean retention time of digesta and in the quantity of digesta have been found to be greater as the level of intake increased (Margan et al. 1982).

Grovum & Williams (1977) noted that the heritability of the rate of turnover of reticulo-rumen contents had not been examined. This still applies. There is no direct evidence of genetically based differences between animals in the regulation of the inputs to and outputs from the reticulo-rumen.

The factors (Section 1.1.2) which may possibly contribute to the susceptibility of an animal to bloat include many that involve regulation of the inputs to and outputs from the reticulo-rumen, and that may affect the quantity of digesta. Studies on saliva production of cows of high and low susceptibility suggest that there may be genetically-based differences in the quantity and protein composition of saliva, but these differences have yet to be related to differences in digesta characteristics or fluid flows from the reticulo-rumen. The following study provides data on some aspects of digestive characteristics of cows differing in susceptibility to bloat.

CHAPTER 2

STUDIES ON THE FOOD INTAKE, MILK PRODUCTION, AND DIGESTIVE AND METABOLIC EFFICIENCY OF COWS DIFFERING IN SUSCEPTIBILITY TO BLOAT

2.1 INTRODUCTION

Susceptibility to bloat has been the only criterion of selection used to establish the herds of HS and LS cows at Ruakura. Differences in susceptibility to bloat may be due to or associated with differences in digestive or metabolic characteristics. These in turn may be reflected as differences in intake, digestion and milk production. Some data on the characteristics of milk production of the two herds are available (Cockrem *et al*. 1983), but no information has been obtained on food intake and digestive or metabolic efficiency of the HS and LS cows. This chapter describes two experiments aimed at providing some of these data. HS and LS cows were compared at two physiological states (pregnant but not lactating in Experiment 1, and not pregnant but lactating in Experiment 2), and when offered 2 diets differing in quality. Physiological state and diet are known to have marked effects on intake and digestion and thus their study may highlight any differences in digestive physiology that exist between cows differing in susceptibility to bloat.

2.2 MATERIALS AND METHODS

2.2.1 Cows

Each experiment involved 16 HS and 16 LS Friesian-Jersey crossbred cows. Half of each susceptibility group was born in 1978 and half born in 1979, and thus were approaching (Experiment 1) or in (Experiment 2) their second or third lactation. Twenty-seven cows were common to both experiments.

Experiment 1 started 87 days before and Experiment 2 ninety-five days after the mean calving date.

The average scores for susceptibility to bloat for the HS and LS groups (Table 2.1) are based on a minimum of 2 seasons' scores, with at least 6 scores taken within a season on days when more than 20% of the HS herd were visually assessed to be grade 2 or above using the scale of Johns (1954) of 0 (no bloat) to 4 (severe bloat requiring treatment to prevent death). The grades were standardised between years by a regression adjustment which ranked each cow's score relative to an HS herd mean value of 2.

Table 2.1 Average (± standard error) susceptibility scores and the proportion of Friesian in the HS and LS cows.

	Experiment 1		Experiment 2	
	HS	LS	HS	LS
Score	2.20±0.11	0.79±0.08	2.15±0.12	0.84±0.07
Friesian (%)	39±6	40±5	35±6	40±5

Each of the founder cows and bulls used to establish the herds were classified for breed (Jersey, Friesian or crossbred) except for some founder cows that were of unknown breed. The breed composition of subsequent progeny (Table 2.1) were based on the contributions from the founder animals through successive matings of purebred and crossbred animals. Purebred Friesian and Jersey bulls were used in alternate years in the first 4 years of the breeding programme (calvings 1973-1976) except that yearling bulls bred in the programme were mated to yearling heifers from 1974 onwards. Most of the bulls bred in the programme were crossbred.

2.2.2 **Diets**

Two diets were offered in each experiment.

Experiment 1:	Pa	Freshly cut herbage consisting predominantly of perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.).
	H	Ryegrass-white clover pasture hay, harvested and baled 5 months previously in spring 1981.
Experiment 2:	Pa	As for Experiment 1.
	PaH	75% Pa and 25% chaffed ryegrass-white clover pasture hay, harvested and baled 12 months previously in spring 1981.

2.2.3 **Experimental design**

Each experiment consisted of two 21-day periods, with 8 HS and 8 LS cows on each diet in the first period, diets being changed over in the second period.

Each 21-day period consisted of 7 days adaptation followed by a 14-day experimental period.

During the adaptation period the cows were individually housed in 2 x 10 m covered pens. Twenty cows remained in these pens during the experimental period. The remaining 12 cows (3 HS and 3 LS per diet) completed 10 days of the experimental period in digestibility stalls that allowed the separate collection over 7 days of faeces and urine. During 4 of the 7 days, 4 of the 12 cows (1 HS, 1 LS per diet) were housed in indirect open-circuit calorimeter chambers that provided metabolic as well as digestibility data. The terms feeding pens, digestibility stalls and chambers are used here to distinguish between the three facilities. The cows involved in digestibility and calorimetry measurements were fully accustomed to the procedures involved.

This design provided data on the daily intake of each diet by 32 cows, water intake and digestibility of each diet by 12 cows, and metabolic efficiency of 4 cows.

2.2.4 Feeding and sampling procedures

Cows in feeding pens

Feeding (a) Experiment 1: Each diet was offered twice daily at 0800 and 1600 hours (h) with sufficient food offered to allow a 15% refusal by each cow at each feeding period. The pasture was cut twice daily using a flail harvester. Food and water were available continuously.

(b) Experiment 2: Pa - as for Experiment 1.

PaH - The hay was offered at 0800 h and pasture at 1100 and 1600 h. The quantity of hay offered was initially set at 3 kg per cow, then adjusted for each cow to 25% of the total daily dry matter (DM) intake, based on 3-day averages. At 1100 h, any remaining hay was mixed with an amount of pasture equivalent to 35% of the expected daily DM intake. The fresh weight of this quantity of pasture DM was estimated using a "quick-dry" DM estimate (Ohaus quick drier). At 1600 h, 65% of expected intake was offered. This procedure ensured that all of the hay was eaten but may have compromised _ad libitum_ feeding.

Sampling Each day food samples were obtained while food boxes were filled, and analysed for DM content. Uneaten food from cows on Pa and PaH diets was removed, weighed and sampled for DM content twice daily, and for cows on H, once daily.

Cows in digestibility stalls and chambers

Feeding (a) Experiment 1: Food was offered as in the feeding stalls but was removed at 2100 h. Water was available continuously.

(b) Experiment 2: As for Experiment 1 except that on PaH, any hay residues were removed at 1100 h.

Sampling Samples taken during the filling of food boxes and aliquot proportions of each cow's refusals were each frozen and combined over the 7-day collection period and subsampled for analysis.

Faeces and urine Faeces and urine were collected daily, weighed, and daily aliquots of each for each cow were bulked over 7 days. The equipment used to separate the faeces and urine was a modified version of that described by Hughes (1963). A portion of the wet faeces sample was retained for nitrogen analysis, and the remainder was oven dried for further analysis. Urine was acidified with 6M HCl to pH 1 and stored at 4 °C until analysed.

Chamber gas collection The design and operation of the chambers have been described by Bryant et al. (1977). Consumption of oxygen (O_2) and production of carbon dioxide (CO_2) and methane (CH_4) were based on the analysis of samples of the ingoing and outgoing air collected over 21-22 h each day.

Milking and weighing Cows were milked at 0700 and 1500 h. The yield of milk was measured at each milking, and the composition was determined weekly on aliquot samples from 4 consecutive milkings for cows in feeding pens. Samples were preserved with Lactabs (pellets containing $K_2Cr_2O_7$, Thompson & Capper Ltd., Liverpool). For cows in digestibility stalls and chambers, aliquot samples of milk were bulked over 2 successive periods of 3 and 4 days and were preserved as above. In both experiments the cows were weighed before feeding at 0800 h on 3 consecutive days at the start of each experimental period, and on 1 day after 16 h fasting at the end of each experimental period. Fasted live weights only are presented in the results.

2.2.5 Analytical procedures

Sample composition was determined as follows:

DM content of foods and residues by oven drying at 100 °C for 24 h. Frozen food samples were sawn and dried at 70 °C for 36 h, for subsequent analysis.

Nitrogen concentration of dried foods and residues, wet faeces, and freeze-dried milk and urine by Kjeldahl digestion.

Energy concentration of dried foods, residues and faeces and freeze-dried milk and urine by adiabatic bomb calorimetry.

Modified acid detergent (MAD) fibre concentration of dried foods by the method of Clancy & Wilson (1966).

Minerals - Magnesium (Mg), calcium (Ca), sodium (Na), and potassium (K) concentrations of dried foods, residues and faeces and freeze-dried milk by flame photometry using a 4 channel flame photometer (a modification of that described by Clinton 1967). Fresh urine was analysed using atomic absorption spectrometry. Phosphorus (P) on all samples was analysed after micro-Kjeldahl digestion by the phospho-molybdo-vanadate procedure (Snell & Snell 1945).

Carbon concentration of dried foods and faeces and freeze-dried milk and urine using a Leco induction furnace followed by gaseous absorption.

CO_2 concentration of urine by the method of Vogel (1958).

Fat, protein and lactose contents of milk by infrared analysis.

Table 2.3 Fasted live weights (kg) of non-lactating HS and LS cows when offered pasture (Pa) or hay (H) in Experiment 1 and of lactating cows when offered Pa or pasture plus hay (PaH) in Experiment 2.

	Pa		H or PaH									
	HS	LS	HS	LS	sed[a]	sig.	Pa-H or Pa- PaH[b]	sed	sig.	LS-HS[c]	sed	sig.
Non-lactating	360	379	371	394	15	ns	-13	1	***	21	15	ns
Lactating	327	365	329	365	14	ns	-1	1	ns	37	14	*

[a] Standard error of the difference for the interaction between susceptibility group and diet.
[b] The difference between the diets (Pa minus H or Pa minus PaH) when averaged for all cows.
[c] The difference between the susceptibility groups (LS minus HS) when averaged for both diets.

Table 2.2 Energy content (MJ/kg DM) and concentrations (g/100 g DM) of MAD fibre, nitrogen (N) and minerals in pasture (Pa) and hay (H).

	Experiment 1				Experiment 2			
	Pa	se[a]	H	se	Pa	se	H	se
Energy	18.19	0.21	17.91	0.08	18.35	0.41	17.61	0.31
MAD fibre	22.69	1.01	33.11	1.58	25.59	0.66	36.58	0.60
N	3.25	0.08	1.75	0.13	2.93	0.01	1.80	0.04
Ca	0.59	0.03	0.64	0.03	0.70	0.05	0.70	0.00
Mg	0.21	0.03	0.20	0.02	0.21	0.01	0.17	0.01
Na	0.21	0.03	0.26	0.04	0.24	0.03	0.23	0.01
K	3.46	0.40	1.83	0.13	3.07	0.41	2.17	0.06
P	0.37	0.02	0.28	0.04	0.25	0.11	0.17	0.05

[a] Standard error for the average of 2 samples

Gas analysis CO_2 and CH_4 by a Beckman 315A infrared analyser, and O_2 by a Beckman G2 paramagnetic analyser.

2.2.6 Statistical analysis

Analysis of variance models (Genstat V, Lawes Agricultural Trust, Rothamsted Experimental Station) were used to test for differences between susceptibility groups and diets.

The standard error of the difference between means (sed) of susceptibility groups, diets or the interaction between susceptibility group and diet is given in this and subsequent chapters, with the following symbols to indicate level of significance:

ns = not significant; + = $P<0.10$; * = $P<0.05$;
** = $P<0.01$; *** = $P<0.001$.

2.3 RESULTS

2.3.1 Diet composition

Hay diets were lower in nitrogen and higher in MAD fibre than pasture diets (Table 2.2). Concentrations of minerals in the foods were similar except hay tended to be lower in K than pasture.

2.3.2 Live weight, food intake and yield and composition of milk from cows in feeding pens

Live weight The LS cows were 21 kg heavier than the HS cows when not lactating, the difference increasing to 37 kg during lactation (Table 2.3). The HS and LS cows were, respectively, 38 and 22 kg lighter during lactation than 6 months previously. Live weights were significantly higher on H than on Pa in Experiment 1, but were similar on each diet in Experiment 2.

Table 2.5 Milk composition and daily yields of milk and its constituents from HS and LS cows offered pasture (Pa) or pasture plus hay (PaH).

	Pa		PaH									
	HS	LS	HS	LS	sed[a]	sig.	Pa-PaH[b]	sed	sig.	LS-HS[c]	sed	sig.
Milk (kg)	13.3	15.4	12.3	14.3	1.0	ns	1.1	0.1	***	2.1	1.0	*
Fat (%)	5.21	4.97	5.05	4.88	0.20	ns	0.13	0.03	**	-0.21	0.19	ns
Protein (%)	3.75	3.57	3.66	3.48	0.10	ns	0.09	0.01	***	-0.18	0.10	+
Lactose (%)	4.99	4.85	4.99	4.88	0.08	ns	-0.02	0.01	ns	-0.13	0.08	ns
Fat (g)	685	755	610	690	44	ns	70	8	***	75	43	ns
Protein (g)	494	546	442	494	32	ns	52	5	***	52	31	ns
Lactose (g)	666	745	611	698	50	ns	51	7	***	83	50	ns

a,b,c see Table 2.3

Table 2.4 Daily intakes of DM by non-lactating HS and LS cows when offered pasture (Pa) or hay (H) in Experiment 1 and by lactating cows when offered Pa or pasture plus hay (PaH) in Experiment 2.

	Pa		H or PaH				Pa-H or					
	HS	LS	HS	LS	sed[a]	sig.	Pa-PaH[b]	sed	sig.	LS-HS[c]	sed	sig.
Non-lactating												
kg/cow	9.1	9.7	8.5	9.0	0.6	ns	0.7	0.1	***	0.6	0.5	ns
g/kg $LW^{0.75}$	110	113	100	102	5	ns	11	2	***	3	5	ns
Lactating												
kg/cow	13.7	14.8	13.0	13.9	0.6	ns	0.8	0.1	***	1.0	0.6	ns
g/kg $LW^{0.75}$	178	178	168	167	6	ns	11	1	***	-1	5	ns

a,b,c see Table 2.3

Food intake Differences between susceptibility groups in daily DM intake, when expressed on an absolute (kg/cow) or metabolic live weight (g/kg $LW^{0.75}$) basis, were small and not significant in either experiment (Table 2.4). Both susceptibility groups consumed 27% of the PaH DM intake as hay. Intakes of Pa were significantly higher than those of H or PaH.

Milk production LS cows produced more milk than HS cows on both diets (Table 2.5). There were no significant differences between susceptibility groups in yields of fat, protein or lactose or in milk composition, except that LS cows produced milk of a slightly lower protein content than did HS cows.

The inclusion of hay in the diet significantly decreased milk yield, percent of fat and protein, and yields of fat, protein and lactose (Table 2.5).

2.3.3 Food intake and digestibility of diets by cows in digestibility stalls

Food intake The intake of DM was lower for the 12 cows when in digestibility stalls than when in feeding pens, and when compared to the intakes averaged for all cows over the experimental periods (Table 2.6, compared to Table 2.4). Relative differences in intake between diets and susceptibility groups, when expressed on a metabolic live weight basis, were similar for the cows in digestibility stalls and when averaged for all cows. When expressed per cow, intakes were significantly higher for the LS cows than the HS cows in both experiments. In Experiment 1 there was a significant interaction between susceptibility group and intake expressed either per cow or per kg $LW^{0.75}$, the LS cows consuming more DM than the HS cows when offered pasture but not when offered hay.

Table 2.6 Daily DM intake, water drunk and apparent digestibilities (%) of DM, organic matter (OM), nitrogen (N) and energy (E) when HS and LS cows were offered pasture (Pa), hay (H) or pasture plus hay (PaH).

	Pa		H or PaH									
	HS	LS	HS	LS	sed[a]	sig.	Pa-H or Pa-PaH[b]	sed	sig.	LS-HS[c]	sed	sig.
					Non-lactating; Experiment 1							
DM intake												
kg/cow	7.2	8.7	7.2	7.6	0.5	*	0.6	0.2	*	1.0	0.4	*
g/kg $LW^{0.75}$	90	98	86	82	5	*	10	3	**	2	4	ns
Water drunk												
kg/cow	3.2	3.7	34.7	35.8	3.6	ns	-31.8	2.5	***	0.8	2.6	ns
kg/kg DM	0.44	0.42	4.81	4.74	0.45	ns	-4.35	0.33	***	-0.05	0.30	ns
Digestibility coefficients												
DM	74.3	73.3	53.6	55.0	0.9	*	19.5	0.4	***	0.2	0.8	ns
OM	76.6	76.8	55.6	57.0	1.1	ns	20.4	0.5	***	0.8	1.0	ns
N	72.0	71.0	49.8	51.8	2.0	ns	20.7	0.8	***	0.5	1.9	ns
E	73.5	72.3	52.1	53.6	1.1	*	20.1	0.5	***	0.2	1.0	ns
					Lactating; Experiment 2							
DM intake												
kg/cow	12.3	14.5	11.3	13.6	0.7	ns	10.0	0.2	***	2.3	0.7	**
g/kg $LW^{0.75}$	156	165	143	153	9	ns	13	2	***	10	9	ns
Water drunk												
kg/cow	11.8	15.7	17.8	25.5	2.9	*	-7.9	0.8	***	5.8	2.8	+
kg/kg DM	0.95	1.07	1.55	1.86	0.19	ns	-0.70	0.08	***	0.22	0.18	ns
Digestibility coefficients												
DM	71.2	71.2	68.3	68.1	0.6	ns	3.0	0.3	***	-0.1	0.5	ns
OM	73.9	73.7	70.6	69.9	0.6	ns	3.6	0.3	***	-0.5	0.5	ns
N	64.5	64.5	65.2	66.3	1.2	ns	-1.3	0.7	+	0.6	1.1	ns
E	69.2	69.3	66.7	66.4	0.7	ns	2.7	0.3	***	-0.1	0.6	ns

a,b,c see Table 2.3

The proportion of the DM consumed as hay on the PaH diet was more variable for cows in digestibility stalls than for the cows in the feeding stalls due to the feed routine adopted when in the digestibility stalls. HS and LS cows consumed 14.4 and 20.2% of DM as hay, respectively.

The quantity of water drunk was similar for both susceptibility groups on each diet in Experiment 1 and on Pa in Experiment 2, but LS cows drank more water than HS cows on PaH (Table 2.6). When expressed per kg DM intake, the quantity of water drunk was similar for both susceptibility groups on all diets. Food intake was significantly lower on H than on Pa in Experiment 1, and on PaH than on Pa in Experiment 2.

Milk yields were slightly higher for the LS cows and lower for the HS cows when in digestibility stalls than when averaged for all of the cows (LS-HS = 4.8, sed 1.8, $P<0.05$ for cows in digestibility stalls). Trends in the composition of milk were similar to those given for all cows so the data are not presented.

Digestibility of the diets Differences between susceptibility groups in the apparent digestibility of DM, organic matter (OM), nitrogen (N) and energy (E) were small and not significant (Table 2.6).

Apparent digestibility values were significantly lower for H than for Pa in Experiment 1. Inclusion of 14-20% hay in the diet of lactating cows decreased the digestibility of DM, OM and E by 3 percentage units, but increased N digestibility by 1.3 percentage units.

Mineral intake and excretion of minerals in urine and faeces are shown in Tables 2.7 and 2.8 for non-lactating and lactating cows respectively. Mineral intakes reflect DM intake and the concentration of minerals in the diet and were generally higher for LS than HS cows,

Table 2.8 Daily mineral intake (I, g) and output, expressed as a percent of intake, in faeces (F), urine (U), milk (M) or retained (R) by HS and LS cows offered pasture (Pa) or pasture plus hay (PaH).

	Pa		PaH									
	HS	LS	HS	LS	sed^a	sig.	Pa-PaH^b	sed	sig.	LS-HS^c	sed	sig.
Na												
I	29.5	35.7	26.9	32.0	2.1	ns	3.2	0.7	**	5.7	1.9	*
F	38.6	38.4	30.8	31.8	8.1	ns	7.2	3.9	+	0.4	7.2	ns
U	35.2	21.1	32.8	29.5	10.6	ns	-3.0	7.6	ns	-8.7	7.4	ns
M	12.9	14.8	12.6	15.7	1.3	ns	-0.3	0.5	ns	2.5	1.2	+
R	13.3	25.7	23.8	23.0	9.4	ns	-3.9	6.5	ns	5.8	6.8	ns
K												
I	376	441	332	392	23	ns	47	7	***	63	22	*
F	15.5	13.0	14.7	11.0	1.6	ns	1.4	0.9	ns	-3.1	1.3	*
U	72.7	72.1	60.1	80.3	9.5	ns	2.2	6.2	ns	9.8	7.2	ns
M	4.5	6.3	4.4	6.3	0.8	ns	0.1	0.1	ns	1.9	0.8	*
R	7.3	8.6	20.8	2.4	9.7	ns	-3.7	6.5	ns	-8.7	7.3	ns
Mg												
I	24.4	28.8	22.2	26.3	1.5	ns	2.4	0.4	***	4.3	1.4	*
F	84.5	87.4	90.6	93.7	6.5	ns	-6.2	5.1	ns	3.0	4.1	ns
U	11.8	11.1	9.8	9.6	1.3	ns	1.8	0.5	**	-0.5	1.2	ns
M	4.6	5.3	5.6	5.4	0.8	ns	-0.6	0.3	ns	0.3	0.8	ns
R	-0.9	-3.8	-6.0	-8.7	6.7	ns	4.9	5.0	ns	-2.8	4.4	ns
Ca												
I	84.8	100.7	79.2	94.7	5.0	ns	5.8	1.4	**	15.7	4.8	**
F	66.8	68.6	73.1	73.9	2.2	ns	-5.8	1.2	***	1.3	1.9	ns
U	0.3	0.2	0.3	0.2	0.1	ns	0.0	0.1	ns	-0.1	0.1	ns
M	14.0	15.9	13.6	15.9	1.7	ns	0.2	0.3	ns	2.1	1.7	ns
R	18.9	15.3	13.0	10.0	2.5	ns	5.6	1.4	**	-3.3	2.0	ns
P												
I	29.6	36.3	25.9	31.3	2.4	ns	4.4	1.3	**	6.1	2.0	*
F	119.8	98.2	106.0	88.9	22.8	ns	11.6	17.7	ns	-19.4	14.5	ns
U	0.7	0.7	0.9	0.9	0.2	ns	0.2	0.2	ns	0.0	0.1	ns
M	57.5	53.7	44.7	56.6	11.6	ns	5.0	7.2	ns	4.1	9.1	ns
R	-78.0	-52.6	-51.6	-46.4	31.2	ns	-16.3	23.3	ns	15.3	20.7	ns

a,b,c see Table 2.3

Table 2.7 Daily mineral intake (I, g) and output, expressed as a percent of intake, in faeces (F), urine (U), or retained (R) by HS and LS cows offered pasture (Pa) or hay (H).

	Pa		H									
	HS	LS	HS	LS	sed^a	sig.	Pa-H^b	sed	sig.	LS-HS^c	sed	sig.
Na												
I	14.0	17.4	18.8	19.8	1.8	ns	-3.6	0.7	***	2.2	1.6	ns
F	44.2	41.3	77.4	73.6	8.2	ns	-32.8	4.8	***	-3.4	6.7	ns
U	41.1	36.4	29.2	38.8	8.3	*	4.8	2.9	ns	2.5	7.8	ns
R	14.7	22.3	- 6.6	-12.4	10.9	ns	28.1	6.6	**	0.9	8.7	ns
K												
I	246	296	133	141	21	*	134	8	***	29	19	ns
F	12.7	9.0	22.5	15.5	2.1	ns	-8.2	1.5	***	-5.4	1.6	**
U	82.4	82.5	79.8	88.9	4.6	*	-1.9	1.6	ns	4.6	4.4	ns
R	4.9	8.5	-2.3	-4.4	4.2	ns	10.0	1.9	***	0.7	3.8	ns
Mg												
I	14.1	17.2	14.5	15.1	1.1	+	0.9	0.6	ns	1.9	0.9	+
F	77.3	82.9	91.0	87.6	4.4	ns	-9.2	3.3	*	1.1	2.8	ns
U	10.8	9.9	12.9	12.5	1.0	ns	-2.4	0.7	**	-0.7	0.7	ns
R	11.9	7.2	-3.9	-0.1	4.3	ns	11.6	3.0	**	-0.5	3.0	ns
Ca												
I	41.1	48.6	45.5	47.6	2.6	+	-1.7	1.4	ns	4.8	2.3	+
F	65.3	69.2	86.4	83.2	5.7	ns	-17.6	4.0	**	0.4	4.0	ns
U	1.9	1.8	0.9	0.5	0.6	ns	1.2	0.3	**	-0.3	0.5	ns
R	32.8	29.0	12.7	16.3	5.5	ns	16.3	4.0	**	0.0	3.9	ns
P												
I	25.9	31.6	19.9	21.2	1.8	*	8.2	0.7	***	3.5	1.6	+
F	88.6	91.0	89.0	92.2	4.4	ns	-0.8	2.7	ns	2.8	3.4	ns
U	0.7	0.8	0.4	0.5	0.1	ns	0.3	0.02	***	0.1	0.1	ns
R	10.7	8.2	10.6	7.3	4.4	ns	0.5	2.8	ns	-2.8	3.5	ns

a,b,c see Table 2.3

and, with the exceptions of Na and Ca in Experiment 1, were higher for Pa than for H or PaH. The proportions of minerals excreted in the urine and faeces were variable, particularly when comparing susceptibility groups, as indicated by the high standard errors of the differences between means.

There were no differences between susceptibility groups in the proportion of Na excreted in urine or faeces or retained, in either experiment, although there was a significant interaction between non-lactating susceptibility groups and diet, the LS cows excreting more Na in the urine than HS cows when offered H, but less when offered Pa. Lactating LS cows excreted a slightly higher proportion of Na in milk than HS cows, reflecting differences in milk yield rather than in the concentration of Na in the milk.

HS cows excreted a higher proportion of K in the faeces than LS cows in both experiments. Non-lactating LS cows excreted significantly more K in the urine than did HS cows on H, but not on Pa. A similar trend occurred with lactating cows, but the differences were not significant. The proportion of K excreted in milk was higher for LS than for HS cows.

There were no differences in either experiment between susceptibility groups in the proportions of Ca, Mg or P excreted or retained.

In Experiment 1, higher proportions of Na, K, Mg and Ca, but not P, were excreted in the faeces of cows offered H than those offered Pa. Urinary excretion of Mg was higher and that of Ca and P lower, on H than on Pa. Greater proportions of Na, K, Mg and Ca were retained on Pa than on H; more Na, K and Mg was excreted than consumed when the cows were offered H.

Table 2.9 Partitioning (% of intake) of energy consumed (MJ/day) by HS and LS cows offered pasture (Pa), hay (H) or pasture plus hay (PaH).

	Pa		H or PaH									
	HS	LS	HS	LS	sed[a]	sig.	Pa-H or Pa-PaH[b]	sed	sig.	LS-HS[c]	sed	sig.
					Non-lactating; Experiment 1							
Intake	140	164	128	152	20	ns	12	7	ns	24	19	ns
Partitioning												
Faeces	26.4	28.6	49.1	46.6	2.3	ns	-20.4	1.0	*	-0.2	2.1	ns
Urine	5.6	5.4	3.6	3.8	0.4	ns	1.8	0.3	ns	0.0	0.3	ns
CH_4	7.0	6.7	6.7	6.3	0.8	ns	0.4	0.2	ns	-0.4	0.8	ns
Heat	52.5	51.9	47.1	46.0	4.6	ns	5.7	1.0	ns	-0.9	4.5	ns
Tissue	8.5	7.4	-6.5	-2.7	4.4	ns	12.6	1.8	+	1.3	4.1	ns
					Lactating; Experiment 2							
Intake	234	276	209	262	33	ns	20	2	+	48	33	ns
Partitioning												
Faeces	30.3	30.8	32.8	33.5	1.0	ns	-2.6	0.5	ns	0.6	0.9	ns
Urine	5.0	4.9	4.9	4.5	0.4	ns	0.3	0.2	ns	-0.3	0.3	ns
CH_4	6.4	6.2	6.7	6.2	0.3	ns	-0.2	0.1	ns	-0.4	0.2	ns
Heat	36.0	35.1	36.8	34.7	0.9	ns	-0.2	0.1	ns	-1.5	0.9	ns
Milk	19.5	21.4	19.1	22.0	1.2	ns	-0.1	0.3	ns	2.4	1.2	ns
Tissue	2.8	1.6	-0.3	-0.9	1.3	ns	2.8	0.7	ns	-0.9	1.1	ns

a,b,c see Table 2.3

In Experiment 2, the proportion of Na in the faeces was significantly lower and that of Ca higher on PaH than on Pa. A greater proportion of Mg was lost in the urine on Pa than on PaH. There was no effect of diet on the proportions of minerals in milk or retained, except for Ca where a greater proportion was retained on Pa than on PaH. More Mg and P was excreted than consumed on both diets.

2.3.4 **Metabolic efficiency of cows in chambers**

There were no differences in either experiment between susceptibility groups in the partitioning of the energy consumed into faeces, urine, CH_4, heat, tissue, or milk (Table 2.9). There were no interactions between diet and susceptibility group in partitioning. A greater proportion of the energy consumed appeared in the faeces and less was retained in tissue on H than on Pa.

There were no differences between susceptibility groups in the partitioning of nitrogen consumed into faeces, urine, milk or tissue, or of carbon into faeces, urine, CH_4, CO_2, milk or tissue, and these data are not presented.

2.4 DISCUSSION

The reduction in the average susceptibility score for LS cows in the Ruakura selection programme was rapid and clearcut within 3-4 years of the programme's start; the cows used in Experiments 1 and 2 were at or near the maximal difference in grade achieved between the susceptibility groups (see Figure 1.1). Furthermore, the differences in milk yield and composition observed between the susceptibility groups in Experiment 2 are consistent with those previously observed over all of the HS and LS cows (Cockrem _et al_. 1983). It is therefore likely that the productions of the cows used here were representative of the herds.

The important results from the 2 experiments are:

(1) Intakes of diets differing in digestibility were similar for both susceptibility groups when expressed on a live weight basis.

(2) Differences in milk yield and composition were small.

(3) There were no differences between susceptibility groups in apparent digestive efficiency when either lactating or not lactating.

(4) Partitioning of energy and other nutrients was similar for both susceptibility groups, although only a small number of cows were used in this study.

These results suggest that if the HS and LS cows differed in digestive function, the differences were insufficient to affect performance. However, interpretation of these results may have been confounded by the effects of breed and by cow quality.

The precise breed composition of individual cows is uncertain. Each cow had a different contribution from founder cows, founder bulls, and crossbred cows and bulls. The estimated proportion of Friesian in the HS and LS cows (Table 2.1) indicated little difference in the proportions of Jersey and Friesian ancestry when averaged across groups, but there was a wide range between cows. Differences exist between breeds of cattle in the efficiency of milk production, and possibly between Friesian and Jersey cows (Hind 1978), but the expression of differences is affected by diet and feeding regime (Freeman 1975). The comparative performance of Friesians and Jerseys and their crossbreeds when offered pasture has not been determined, although the existence of substantial differences has been proposed (Bryant & Macmillan 1985).

Differences between founder sires and dams in breeding indices (BI) for milk and fat production could have influenced the production data. High BI Jersey cows eat more, produce more and are heavier than low BI Jerseys (Bryant & Trigg 1981), whereas high BI Friesian cows produce more but are lighter than low BI Friesians (Davey _et al_. 1983).

The 6-11% difference in live weight observed in Experiments 1 and 2 could be due to a breed difference or to the influence of the founder animals. Alternatively, selection for low susceptibility may have been associated with anatomical features which resulted in larger and heavier LS cows.

While Cockrem _et al_. (1983) concluded that differences in breed or in BI of the founder bulls were unlikely to have contributed to the differences they observed in the yield and composition of the milk from HS and LS cows, there is uncertainty about this conclusion. Effects of possible differences in breed and/or in BI between susceptibility groups on some or all of the parameters measured may have compensated for a real effect due to susceptibility. It therefore cannot be inferred from the results obtained here that cows differing in susceptibility to bloat do not differ in digestive characteristics.

Cockrem _et al_. (1983) suggested that HS cows have a greater volume of digesta in the reticulo-rumen than do LS cows (Chapter 1.1.10), and that this difference betweeen susceptibility groups in volume occurred on a range of feeds, including pasture and hay (Cockrem, pers. comm.). Diets of relatively low digestibility, such as hay, are associated with an increase in the retention time of digesta in the reticulo-rumen and total digestive tract (Blaxter _et al_. 1961; Campling _et al_. 1961). Retention time in the reticulo-rumen is involved in the regulation of voluntary intake (Blaxter _et al_. 1956, 1961; Campling _et al_. 1961; Ulyatt _et al_. 1967), contributing to the lower intakes observed on hay than on pasture. The proportion of the total DM intake of PaH due to hay was different for the HS and LS cows in Experiment 2, but there was no evidence that this confounded the comparison between susceptibility groups since the energy loss in faeces and the apparent digestibility coefficients were similar for both HS and LS cows on the PaH diet, as they were on the Pa and H diets. The reason for using diets differing in digestibility was to enhance any differences in digestive characteristics that existed between HS and LS cows. No enhancement occurred as there were no interactions between susceptibility group and diet for the parameters measured. However, although the major determinant of apparent digestibility of a diet is its apparent digestibility in the rumen (Ulyatt _et al_. 1967), reduced digestion in the reticulo-rumen may be compensated for by increased digestion in the lower tract. This has been found in comparisons between diets (Ulyatt _et al_. 1967) and between levels of intake (Grovum & Williams 1977). Lack of differences between susceptibility groups in digestibility coefficients do not, therefore, preclude the existence of differences between susceptibility groups in digestibility and retention of digesta in the reticulo-rumen.

Factors other than diet which affect voluntary intake include gut capacity and physiological state (pregnancy, fatness, lactation). The experiments were carried out 6-12 weeks before, and 3-4 months after parturition. Despite the effects on voluntary intake of physical compression by the uterus and possibly endocrine changes during late pregnancy (Forbes 1970), and of lactation (Hutton _et al_. 1964), the data obtained suggest that gut capacity was similar for both susceptibility groups in these two physiological states. However, LS cows drank more water when lactating than did HS cows. Water intakes are generally proportional to DM intakes (Grovum & Williams 1977; Ulyatt *et al*. 1984). The higher milk yield of LS cows may have partly accounted for their higher water intakes, although Owen *et al*. (1968) found that the water intake during lactation of cows offered a dry diet was more closely correlated with DM intake than with milk yield.

Cockrem *et al*. (1983) speculated that factors associated with mineral metabolism may be associated with the difference in volume of digesta they observed between HS and LS cows. For this reason the data on mineral intake and excretion were obtained. Higher faecal K excretion (as a percentage of intake) by HS cows was consistent in both experiments, for all diets. There were interactions between diets and susceptibility groups in the urinary Na and K excretions, although this was only significant during Experiment 1, possibly because the diets were more extreme than those in Experiment 2. Excretion of Na and K in the faeces and urine are regulated through absorption from the gut and through the kidneys in response to their concentrations in body fluids. While the biological significance of the differences observed here is not apparent, it may become so when mineral metabolism is investigated and discussed in more detail (Chapters 6 and 7).

Food intake and milk production in Experiment 2 were measured under non-bloating conditions. Bloat, or the use of chemicals to control bloat, may affect food intake and milk production. A belief that cows which bloat are those which have high production and high food intake or rate of eating has not been verified (Hancock 1953, Johns 1954). It is generally accepted, however, that the occurrence of bloat limits DM intake (Hancock 1953; Johns 1954) and consequently, milk yield. Although some compensatory intake may occur after relief of bloat, large reductions in intake may not be fully compensated for during subsequent feeding. Wright (1971) found that drenching with the bloat preventative Pluronic L-64 increased production from severely bloating cows, but slightly decreased production from mildly bloating cows. The effect on production from bloating cows may have been related to an increased water turnover (Wright & Jones 1974), resulting from increased DM and water intakes.

The type of feeding facility influenced the food intake of the cows. The cows ate less when in the digestibility stalls than when in the feeding pens, and food intakes in both facilities may have been lower than when grazing. This may account in part for the low levels of production achieved by these cows, relative for example to the production of the high BI Friesian and Jersey cows described by Bryant & Macmillan (1985). The cows in the present study were also younger than those described by Bryant & Macmillan (1985). Levels of intake and production higher than those achieved in the work described here may be necessary for any differences in digestive characteristics between the HS and LS cows to become evident.

These aspects should be taken into account when considering the general conclusion that the selection for low susceptibility in this herd of crossbred animals does not appear to have resulted in a less productive animal. Some evidence was obtained that HS and LS cows may differ in electrolyte metabolism. Cockrem _et al_. (1983) have speculated that electrolyte metabolism may be associated with differences observed between HS and LS cows in the volume of digesta in the reticulo-rumen. The subsequent chapter describes more detailed investigations of whether HS and LS cows differed in reticulo-rumen characteristics, with particular emphasis given to defining relationships between susceptibility and the quantity of digesta in the reticulo-rumen.

CHAPTER 3

INVESTIGATION OF THE QUANTITY AND COMPOSITION OF DIGESTA IN THE RETICULO-RUMEN OF COWS DIFFERING IN SUSCEPTIBILITY TO BLOAT

3.1 INTRODUCTION

Cockrem *et al*. (1983) suggested that LS cows have a lower volume of digesta in the reticulo-rumen after a period of fasting than do HS cows. The implications, supported by theoretical calculations based on fermentation studies *in vitro*, were that LS cows could not produce sufficient gas and foam to bloat to the extent observed in HS cows. Experiments involving the exchange of reticulo-rumen digesta between HS and LS cows indicated that LS digesta was capable of causing relatively severe bloat in LS cows, if present in a volume similar to that found in HS cows.

The above study involved only 2 HS and 2 LS non-lactating cows. No information was obtained on the diets consumed prior to the observations on the volume of the digesta, nor was there control or measurement of DM and water intakes by the cows prior to the fasting period. Water was available over the fasting period but the amounts drunk were not measured. It was not established whether the differences in the volume of digesta after fasting arose from differences in volume immediately after feeding, or from differences in digesta loss after feeding. Furthermore, the technique used to assess the volume of digesta was probably influenced by the amount of foam in the digesta. The weight and DM content of digesta were not measured. This chapter describes two experiments aimed at overcoming these limitations. The quantity and composition of digesta were determined in HS and LS cows when not lactating but pregnant (Experiment 3) and when lactating but not pregnant (Experiment 4).

3.2 MATERIALS AND METHODS

3.2.1 Cows

Five HS and 5 LS cows were each fitted with a 10 cm diameter cannula in the dorsal rumen. Information on the cows, including susceptibility grade, age, and breed is supplied in Appendix 1. The cannulae of moulded rubber were obtained from Bar Diamond, Idaho, USA. Four of the cows were fistulated in December 1981 and were those used by Cockrem *et al*. (1983) in their digesta exchange experiments. The remaining 6 cows were fistulated in February 1983, 3 months prior to Experiment 3. The fistulations were carried out by Mr A.M. Day, Veterinarian for the Ruakura Animal Research Station. Some problems were encountered with the fistulations, resulting in varying degrees of leakage in some animals (see later).

Experiment 3 started 125 days before and Experiment 4 sixty days after the mean calving date.

The cows were housed in indoor feeding stalls during feeding and measurement periods, and yarded outside at all other times.

The cows were milked at 0700 h and 1500 h in Experiment 4.

3.2.2 Feeding, experimental design and measurements

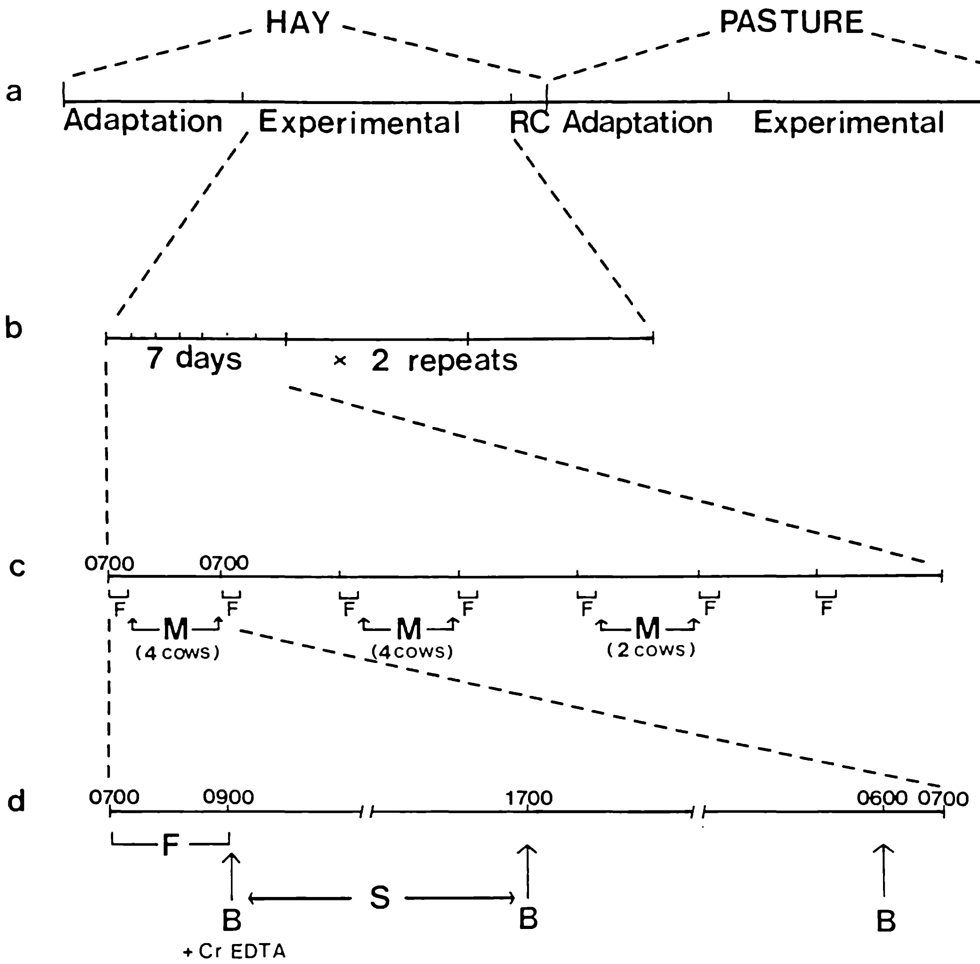
Experiment 3 - Restricted feeding of hay and pasture to non-lactating cows

Diets and feeding

The diets offered were:

Pasture - herbage consisting predominantly of perennial ryegrass and white clover was cut with a flail harvester one month prior to use, and stored at -18 °C.

Hay - chaffed ryegrass-white clover hay.



KEY		
	RC	Reticulo-rumen capacity estimates
	F	2 h feeding period
	M	Measurement period
	B	Bailing
	S	Sampling period (rumen fluid)

Figure 3.1 The schedule of measurements for Experiment 3.

The diets were offered at a maintenance feeding level, calculated from the MAFF (1975) allowance of 0.58 MJ ME per kg $LW^{0.73}$, assuming ME values of 8.0 and 10.0 MJ/kg DM for hay and pasture respectively, and using fasted live weights taken prior to the start of the experiment (see below). These allowances were subsequently reduced by 20% during the adaptation period on both diets, in an attempt to ensure all cows consumed similar quantities of DM per unit live weight.

The frozen pasture was thawed for 24 h prior to feeding. The pasture had been frozen in 9 batches, corresponding to the day of harvest and with each batch sufficient for 5-10 days feeding. Batches were sampled and analysed for DM content prior to use in order to determine the daily fresh weight to be offered.

The hay was chaffed in batches sufficient for 3-4 days feeding and each batch was sampled and analysed for DM content.

Each diet was offered for 2 h each day, at 0700 h. Water was offered for 10 minutes at the end of the 2 h feeding period and also after 1 h feeding when the diet was hay.

Experimental design Each diet was offered for 38 days, consisting of 14 days adaptation followed by a 24-day experimental period (Figure 3.1a). All cows were offered the same diet, the order being hay followed by pasture. During the 24-day experimental period three sets of digesta measurements were made on each cow at 7 day intervals (Figure 3.1b). The cows were divided into 2 groups of 4 cows and 1 group of 2 cows with cows of the same age being grouped together (Figure 3.1c).

Digesta measurements The set of measurements consisted of the following (Figure 3.1d):

(1) The weight and DM content of digesta in the reticulo-rumen were measured immediately after the 2 h feeding period (0 h) and 8 and 21 h later. This procedure, subsequently referred to as bailing, is described in Section 3.2.3.

(2) Chromium complexed with ethylenediamine tetra-acetic acid (Cr EDTA) was added to digesta at 0 h, and the dilution rate of Cr estimated during the subsequent 8 h.

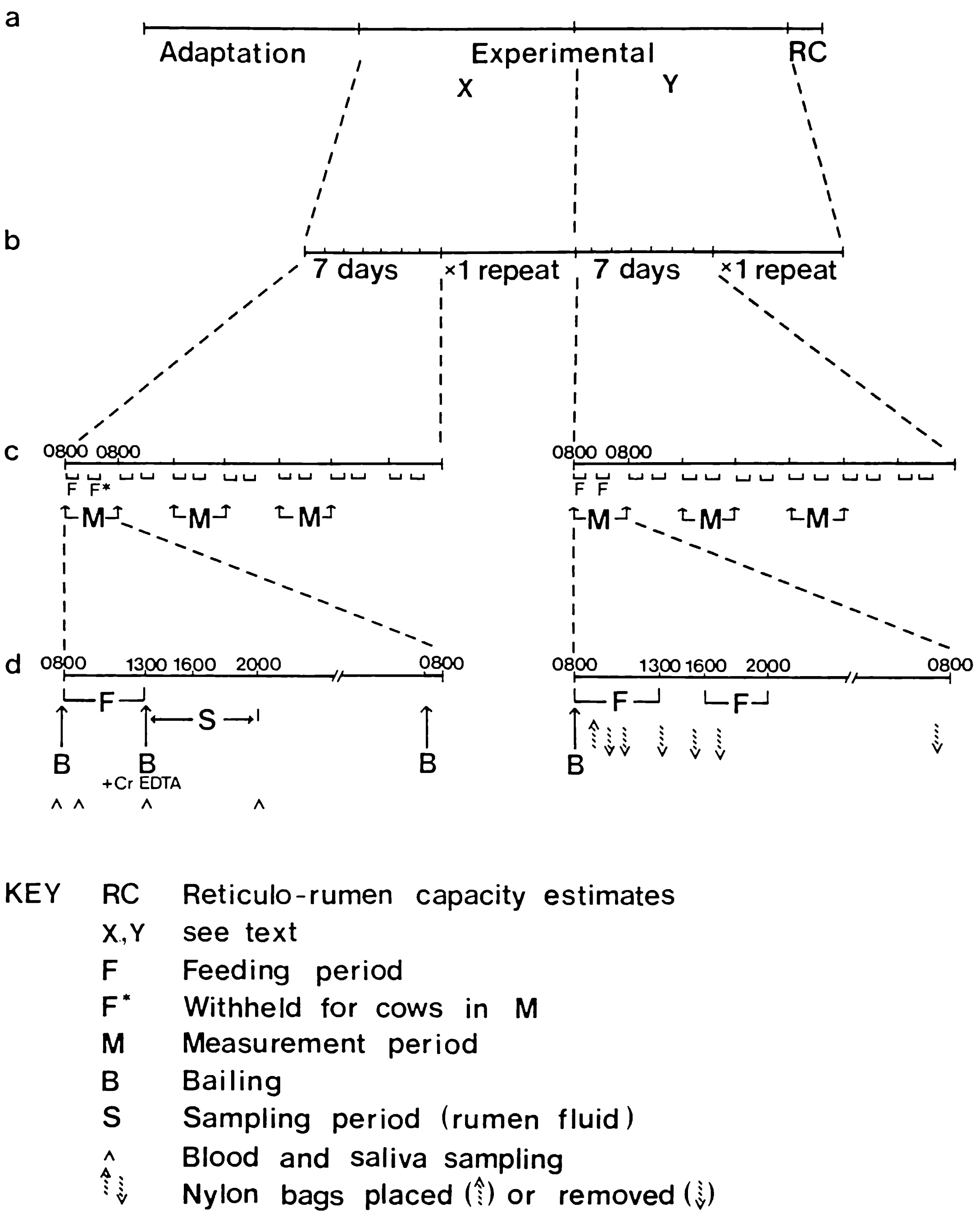
(3) Rumen fluid was sampled from digesta removed at 0, 8 and 21 h, and from digesta *in vivo* at 1-2 hourly intervals between 0 and 8 h, for analysis of osmolality and concentrations of Na, K and Cr.

Reticulo-rumen capacity The capacity of each cow's reticulo-rumen was estimated immediately before offering hay, and both before and after offering pasture, on each of the final 3 days of the experimental periods. Estimates were obtained each day on all 10 cows by the appropriate scheduling of feeding times.

Live weight The cows were weighed on 2 consecutive days prior to the start of the experiment after 2 h grazing followed by 22 h fasting. Subsequently, the cows were weighed before feeding on 3 consecutive days at the start and end of each experimental period.

Cows were subjectively scored on condition or fatness over the pin and hip bones each week on a scale ranging from 0 (very thin) to 10 (very fat).

Modification of diet The concentrations of Na and K in rumen fluid obtained from the cows when offered hay indicated that the cows were in a low Na status. Samples of saliva taken from between the cheek and molars when the cows were on pasture confirmed this. The diet



KEY		
	RC	Reticulo-rumen capacity estimates
	X,Y	see text
	F	Feeding period
	F*	Withheld for cows in M
	M	Measurement period
	B	Bailing
	S	Sampling period (rumen fluid)
	∧	Blood and saliva sampling
	⇡⇣	Nylon bags placed (⇡) or removed (⇣)

Figure 3.2 The schedule of measurements for Experiment 4.

of each cow was subsequently supplemented with 30 g NaCl per day, starting one day after the second set of measurements on pasture had been completed.

Experiment 4 - *Ad libitum* feeding of pasture to lactating cows.

Diets and feeding Pasture consisting predominantly of perennial ryegrass and white clover was cut twice daily using a flail harvester and offered at 110-115% of expected intake from 0800 to 1300 h and from 1600-2000 h each day, with a supplement of NaCl (30 g/cow/day).

Free access to a NaCl block was provided during the 4 months grazing since Experiment 3.

Experimental design A 14-day adaptation period was followed by a 30-day experimental period. The experimental period included two 14-day periods (X, Y, Figure 3.2a).

Measurements of the quantity and composition of digesta and of the composition of blood and saliva were made in Period X. Measurements on the rate of DM degradation in the rumen were made in Period Y. The cows were grouped in Periods X and Y as in Experiment 3. Measurements on each cow were repeated at 7-day intervals during both periods (Figure 3.2b,c).

Measurements

Period X: In each measurement set (Figure 3.2d):

(1) Digesta was bailed immediately before feeding at 0800 h and at 0 and 18 h after feeding. The 1600-2000 h feed was omitted for those cows on which the measurements were being made.

(2) Cr EDTA was added to digesta removed at 0 h.

(3) Rumen fluid was sampled from digesta bailed and at 1-2 hourly intervals for 7 h after Cr EDTA addition, and analysed for osmolality and concentrations of Na, K and Cr.

(4) Digesta pH was recorded in the rumen and reticulum just prior to each digesta removal time and at 3, 5 and 7 h after feeding.

(5) Blood and saliva were sampled before feeding, 15-25 minutes after the start of feeding, and at 0 and 7 h after feeding. The volume of saliva collected in 5 minutes was measured and subsamples were analysed for osmolality and concentrations of total protein, Na, K and CO_2.

The packed cell volume of blood and the osmolality and concentrations of Na and K in plasma were determined.

Period Y: In each measurement set (Figure 3.2d), swallowed boli of food and saliva were collected at the cardia over 15 minutes when food was offered at 0800 h. Samples of the collected material were placed in nylon bags and inserted into the rumen. Feeding resumed (0900 h) and bags were removed at 1, 2, 4, 6, 8 and 22 h after insertion. Collected boli and the material remaining in the bags were analysed for DM content and the concentration of nitrogen.

Reticulo-rumen capacity The capacity of each cow's reticulo-rumen was estimated immediately before feeding at 0800 h on days 29 and 30 of the experimental period.

Live weights The cows were weighed at 0730 h on 3 consecutive days at the start and the end of the experiment. Condition scores were estimated each week.

3.2.3 Techniques and sampling procedures

Foods and residues

Experiment 3

Foods were sampled daily as food boxes were filled, and analysed for DM content. A sample was frozen daily and bulked over each of the three 7-day periods during which measurements were made, for subsequent analysis. Residues were individually weighed and sampled for DM content.

Experiment 4 The 2 daily foods were sampled and analysed for DM content and a sample frozen and bulked over the four 7-day periods for subsequent analysis. Residues were removed twice daily, weighed, and analysed for DM content.

Digesta

Bailing Digesta was removed by hand through a tared plastic chute attached to the cannula and leading into a tared metal bin enclosed in polystyrene insulation. Fluid digesta was removed using a 250 ml plastic beaker. The chute, bin, and digesta were weighed before and after the mixing and sampling of the digesta. The volume of digesta was estimated using a calibration based on water volume (ℓ). The bin, chute, and any unreturned digesta were weighed after returning digesta to the rumen.

Sampling

(a) DM: After mixing of digesta, a sample (0.5 to 1.0 kg) was removed from each of the upper, middle, and lower areas in the bin. The samples were removed by hand if digesta was relatively solid, or by using a 2 ℓ plastic beaker if relatively fluid. The 3 samples were combined and mixed and a subsample (0.5 to 1.0 kg) was removed. The remainder was returned to the bin, and the process was repeated for a duplicate sample.

(b) Fluid: (i) Bin contents: Digesta (0.3-0.5 kg) from the upper, middle and lower areas of the bin were expressed through cheesecloth and 50 ml of the fluid obtained was preserved with 1 ml 5% $HgCl_2$ solution. The remaining fluid and pressed digesta were returned to the bin.

(ii) *In vivo* contents: Digesta (0.3-0.5 kg) from each of the reticulum and dorsal, ventral, and cranial rumen was sampled as for bin contents, and the remainder returned to the rumen.

Rumen fluid samples were centrifuged for 15 minutes at 3000 rpm and the supernatant stored at 4 °C for analysis.

Dilution rate

About 1 ℓ of Cr EDTA solution, containing 2.7 g Cr/ℓ (Binnerts *et al*. 1968), was distributed through the digesta in the bin using a cattle drenching gun with an extended nozzle. The quantity of solution added was determined from the initial and final weights of the gun and fluid pack. This method of addition of Cr EDTA was compared to alternative methods of addition of marker to digesta *in vivo*, in a preliminary investigation of the use of Cr EDTA. For details of this investigation see Appendix 2a,b. Also investigated was the method of sampling of rumen fluid from 3 sites around the rumen, compared to sampling from the reticulum only.

Reticulo-rumen capacity

Digesta was removed from the reticulo-rumen into a covered bin immersed in water at 39 °C. A meteorological balloon was inserted into the empty rumen and reticulum and filled with water at 39 °C. The water was pumped in until the reticulo-rumen was estimated to be about 80% full. Water was then gravity-fed down a clear plastic pipe passing vertically through the cannula into the balloon until the water level in the pipe fluctuated round a level corresponding to the dorsal mid-line in the lumbar region. The water in the balloon was removed, initially by syphoning, and weighed. The total operation including removal and subsequent replacement of digesta took 20-30 minutes per cow.

pH

The *in situ* pH of digesta in the reticulum and the ventral rumen were determined with the aid of a portable pH meter (Digi-Sense, Cole Parmer).

Saliva

Experiment 3: Duplicate samples of saliva were collected with acid washed Na-free swabs placed between the cheek and upper molars near the opening of the duct from the parotid salivary gland. The weight of saliva collected was determined by weighing the swab, in its jar, before and after collection. Electrolytes in the saliva were extracted by the addition of 10 ml of 5% (v/v) HCl.

Experiment 4: Saliva was collected under suction by a bit which sampled both under the tongue in the region of the opening of the ducts of the mandibular gland and above the tongue and close to the opening of the ducts of the parotid gland. Saliva collected over 5 minutes was centrifuged for 10 minutes at 2000 rpm, and the supernatant stored at 4 °C. The 2 saliva collections are subsequently referred to as front (under the tongue) and rear (between cheek and molars).

Blood

Blood (10 ml) was collected from either the tail vein or artery into ammonium heparinised vacutainer tubes. The packed cell volume was determined immediately after withdrawal using a microhaematocrit centrifuge. Plasma was separated by centrifugation for 10 minutes at 2000 rpm, and stored at 4 °C for analysis.

Collection and digestibility in nylon bags of bolus material

Digesta was removed until the level in the reticulum and rumen allowed collection of swallowed bolus material at the cardia without contamination from digesta. Food was offered for 15 minutes and the boli were collected using a webbed glove. There was minimum contact between the hand and cardia. The quantity of food consumed was determined from weights offered and uneaten. Collected bolus material was weighed and subsampled for placement in the bags and for the analysis of DM content and concentrations of nitrogen and minerals.

The nylon bags measured 9 cm x 18 cm (9 x 15 cm when tied) and were double stitched with nylon thread. Specifications of the cloth, supplied by Swiss Screens (Australia) Pty Ltd, were: mesh opening, 44 microns; mesh count, 126.6 per cm; open area, 31%; fabric thickness, 61 microns; weight, 32 g/m^2.

Immediately after bolus collection, subsamples of approximately 20 g were weighed and placed into tared bags, and the bags tied with nylon cord. The cords extended 0.6-0.7 m to the point of attachment to the cannula, and were threaded through plastic tubing (outer diameter 0.5 cm) in groups of 4 to reduce tangling. A 100 g stainless steel weight encircled the lower end of the plastic tube just above the 4 bags. Six tubing lines, or 24 bags, were suspended in the ventral rumen of each cow.

Four bags, or 1 tubing line, were removed at 1, 2, 4, 6, 8 and 22 h, and washed for 2 minutes under a constant water flow before drying and weighing. Dried material was ground for nitrogen analysis.

3.2.4 Analytical procedures

Sample composition was determined as follows:

DM, nitrogen, energy, MAD fibre and mineral concentrations of freeze-dried food samples, and nitrogen concentration of dried bag samples, as described in Section 2.2.5.

Total water-soluble sugar concentration of freeze-dried foods by the anthrone technique (Bailey 1958).

***In vitro* digestibility** of organic matter in freeze-dried foods by the method of Minson & McLeod (1972).

DM content of digesta by oven drying at 100 °C for 36 h.

Osmolality of plasma, rumen fluid and saliva samples within 36 h of sampling by measurement of vapour pressure using a Vapour Pressure Osmometer Model 5100 C (Wescor Inc., USA).

Cr concentration of rumen fluid samples by atomic absorption spectrometry using an oxygen-acetylene flame.

Na and K concentrations of plasma, rumen fluid and saliva samples by atomic absorption spectrometry.

Total protein concentration of saliva samples (Experiment 4) by the method of Lowry *et al*. (1951).

CO_2 concentration of saliva by the method of Conway (1957).

3.2.5 Calculations and statistical analysis

Net fluid, DM and total digesta losses over 0-8 and 8-21 h in Experiment 3 were calculated from the weights of digesta returned and subsequently removed. For example:

$$\text{0-8 h fluid loss (kg)} = (\text{Rem 8F} - \text{Ret 0F}) \times \frac{480}{T}$$

where Rem 8F = fluid (kg) removed at 8 h

Ret 0F = fluid (kg) returned at 0 h

T = time (minutes) elapsed between the start of digesta return at 0 h and the end of digesta removal at 8 h.

The time elapsed for 8-21 h losses was adjusted to 780 minutes. The relationships between the weight of digesta in the rumen at 0 or 8 h and its subsequent loss were investigated by regression analysis. Equations were tested for differences in slope between susceptibility groups. If no difference in slope existed the equations were tested for differences in intercepts at the slope obtained from the average of those for the susceptibility groups. These pooled regression equations were used to recalculate 0-8 and 8-21 h losses by adjusting for the differences between digesta removed and returned at 0 and 8 h (ie. samples, spillage). The estimated loss over 21 h was the sum of the adjusted 0-8 and 8-21 h losses. The relationship between 21 h loss and the quantity of digesta removed at 0 h was obtained as above.

Digesta losses in Experiment 4 were calculated over 0-18 h adjusted to 1080 minutes, as for Experiment 3. Net losses of fluid, DM and total digesta during feeding were calculated as indicated in the following example for fluid.

$$\text{Fluid loss (kg)} = (\text{Pre-feed Ret F} + \text{Cons F} - \text{Post-feed Rem F}) \times \frac{360}{T}$$

where Pre-feed Ret F = fluid (kg) returned immediately before feeding

Cons F = water (kg) consumed during feeding and drinking.

Post-feed Rem F = fluid (kg) removed immediately after feeding.

T = time (minutes) elapsed between the start of digesta return before feeding and the end of digesta removal after feeding.

Fluid dilution rate, D (h^{-1}): The slope of the line relating the natural log (ln) of Cr concentration with time (h) equals -D. In Experiment 3, ln Cr concentration for each cow in each week was plotted against time. In 5 of the 60 individual plots, the residual variance exceeded variance of the y-variate, so these 5 were omitted from subsequent calculations and statistical analysis involving D. In addition, any individual points that deviated from an otherwise

Table 3.1 Energy content (MJ/kg DM), digestibility _in vitro_ (%) and concentrations (g/100 g DM) of MAD fibre, water-soluble sugar, nitrogen (N) and minerals in hay and pasture diets.

	Experiment 3				Experiment 4	
	Hay	se[a]	Pasture	se[a]	Pasture	se[b]
Energy	18.43	0.03	18.85	0.44	17.90	0.15
Digestibility	65.40	1.09	69.39	0.71	66.33	0.46
MAD fibre	30.45	0.47	26.67	0.69	31.49	0.89
Sugar	7.02	0.29	5.17	0.24	4.71	0.89
N	2.08	0.02	3.91	0.08	3.33	0.14
Ca	0.57	0.01	0.89	0.03	0.73	0.04
Mg	0.21	0.01	0.23	0.01	0.27	0.01
Na	0.27	0.01	0.14	0.01	0.20	0.02
K	2.55	0.04	3.34	0.11	3.89	0.12
P	0.26	0.01	0.46	0.01	0.44	0.03

[a] Standard error of the mean of 3 samples
[b] Standard error of the mean of 4 samples

straight line were omitted for the estimation of D. This applied to the first sample time in most instances (for example see Appendix 2b).

The net fluid inflow to the rumen and the outflow to the omasum were calculated (Reid 1965), as follows:

$$\text{Net inflow } (\ell/\text{h}) = \frac{(V_1 - V_2) \cdot D}{\ln (V_1/V_2)}$$

$$\text{Outflow } (\ell/\text{h}) = \text{net inflow} + \frac{V_1 - V_2}{T}$$

where V_1 = fluid volume (ℓ) at start of period when marker added

V_2 = volume (ℓ) at end of period

D = dilution rate

T = time (h)

The weights of fluid estimated by oven drying were assumed to be equivalent to volume (ℓ, V_1 and V_2), without correction for the DM content of the samples of centrifuged rumen fluid upon which the concentration of Cr was determined.

The data were analysed for differences between susceptibility groups and, where appropriate, sample times using analysis of variance models provided by Genstat V.

3.3 RESULTS

3.3.1 **Composition of diets**

The hay offered in Experiment 3 was lower in digestibility _in vitro_ and concentrations of nitrogen, Ca, K and P and higher in concentrations of MAD fibre and Na than the pasture (Table 3.1).

3.3.2 Live weight

Experiment 3 Live weights averaged for the start and end of the experimental period were 390 and 449 kg (sed 32, ns) on hay, and 382 and 428 (sed 34, ns) on pasture, for HS and LS cows, respectively.

Experiment 4 The LS cows were 50 kg heavier than the HS cows (452 and 402 kg, respectively, sed 23, $P<0.10$) during the experimental period.

3.3.3 Intake and the quantity of digesta in the reticulo-rumen

Experiment 3 DM intakes, averaged for the 3 measurement days on each diet for each cow, were similar for both susceptibility groups (Table 3.2). When expressed on a live weight (LW) basis the intakes of hay were 55 and 53 g/kg $LW^{0.75}$ (sed 6, ns) and of pasture were 37 and 34 g/kg $LW^{0.75}$ (sed 6, ns) for the HS and LS cows, respectively. Intakes on measurement days were not significantly different from the averages over the experimental periods. DM intakes were low on pasture due to consistent refusals of food, despite the already low feeding level.

Intakes of water in the food and by drinking (Table 3.2), were similar for HS and LS cows when offered hay. When the diet was pasture, the LS cows consumed 6.9 kg more water than did the HS cows because of a difference in water drunk, since food intakes were similar.

Table 3.2 Daily intakes (kg/cow) of DM and water by HS and LS cows on measurement days (Experiment 3).

	Hay				Pasture			
	HS	LS	sed	sig.	HS	LS	sed	sig.
DM	4.76	5.11	0.44	ns	3.24	3.19	0.35	ns
Water	20.8	21.0	3.5	ns	20.9	27.8	2.7	+

Table 3.4 Pooled regression coefficients (b ± standard error) and intercepts for the loss (kg) of fluid, DM and total digesta over 8 and 21 h on the quantity (kg) present in the reticulo-rumen at 0 or 8 h, for HS and LS cows offered hay and pasture (Experiment 3).

	Time after feeding (h)	b	± se	sig.	Intercepts				
					HS	LS	sed	sig.	R^{2a}
Hay									
Fluid	0-8	-0.004	0.134	ns	9.36	13.30	2.21	ns	0.15
	8-21	0.339	0.063	**	-6.65	-4.92	1.03	ns	0.79
	0-21	0.385	0.139	*	-3.92	0.11	2.30	ns	0.62
DM	0-8	0.167	0.083	+	0.70	0.69	0.19	ns	0.21
	8-21	0.161	0.045	*	1.19	1.50	0.09	*	0.79
	0-21	0.300	0.073	**	1.74	2.03	0.17	ns	0.73
Total	0-8	0.015	0.122	ns	10.15	14.07	2.29	ns	0.15
	8-21	0.322	0.058	**	-5.60	-3.63	1.06	ns	0.80
	0-21	0.374	0.128	*	-2.12	2.21	2.39	ns	0.64
Pasture									
Fluid	0-8	0.341	0.067	**	-10.24	-5.66	1.42	*	0.80
	8-21	0.313	0.082	**	-5.10	0.54	1.21	**	0.76
	0-21	0.550	0.077	***	-13.05	-4.15	1.63	**	0.90
DM	0-8	0.264	0.073	*	-0.43	0.06	0.19	*	0.69
	8-21	0.377	0.056	***	-0.21	0.18	0.11	*	0.85
	0-21	0.533	0.057	***	-0.51	0.20	0.15	**	0.93
Total	0-8	0.329	0.063	**	-10.36	-5.30	1.50	*	0.81
	8-21	0.322	0.077	**	-5.49	-0.56	1.29	**	0.78
	0-21	0.548	0.074	***	-13.53	-3.92	1.74	**	0.91

[a] Percentage of variance accounted for by the regression

The weights of fluid, DM and total digesta removed from the reticulo-rumen at 0 h were similar for both susceptibility groups, on each diet (Table 3.3).

Table 3.3 Quantity (kg) of fluid, DM and total digesta removed from the reticulo-rumen at 0 h for HS and LS cows offered hay and pasture (Experiment 3).

	Hay				Pasture			
	HS	LS	sed	sig.	HS	LS	sed	sig.
Fluid	58.93	61.60	6.06	ns	56.03	56.77	4.45	ns
DM	8.07	8.40	0.72	ns	5.67	5.93	0.70	ns
Total	67.00	70.00	6.77	ns	61.70	62.70	5.12	ns

Less digesta was removed from the reticulo-rumen of cows when offered pasture than when offered hay.

The relationships between the weight of fluid, DM and total digesta in the reticulo-rumen at 0 or 8 h and the subsequent digesta loss, (0-8, 8-21, 0-21 h), are shown by the pooled regression coefficients and intercepts for the average of the 3 weeks data (Table 3.4).

Hay The R^2 values were low during 0-8 h, but were higher for losses over 8-21 and 0-21 h. Except for DM loss over 8-21 h, there were no differences in intercepts between the susceptibility groups. The regression analyses indicated that the loss of digesta from the reticulo-rumen was proportional to the quantity of digesta initially present, and that the proportion lost was similar for both susceptibility groups. Thus for a given quantity of fluid or DM in the reticulo-rumen after feeding, there was no difference between susceptibility groups in the subsequent digesta loss and, therefore, in the quantity of digesta remaining at 21 h (or before the next feeding period). The weights remaining at 21 h, calculated from

Tables 3.3 and 3.4, were 40.16 and 37.77 kg fluid and 3.91 and 3.85 kg DM for HS and LS cows, respectively.

Pasture The R^2 values were higher on pasture than on hay for all time periods. The regression coefficients were, in general, higher on pasture than on hay, indicating that more digesta was lost from the reticulo-rumen per kilogram digesta present on pasture than on hay. For all time periods, and for fluid, DM and total digesta, intercepts were significantly higher for LS cows than for HS cows. This indicated that for any given quantity of digesta in the reticulo-rumen, LS cows subsequently lost more digesta than did HS cows, resulting in a lower quantity of digesta in the reticulo-rumen of LS cows at 21 h. The calculated weights remaining at 21 h were 38.26 and 29.70 kg fluid and 3.16 and 2.57 kg DM for HS and LS cows, respectively.

The regression analyses indicated that differences in the loss of both fluid and DM contributed to the lower weight of digesta in LS cows at 21 h. The DM content of the digesta did not differ between susceptibility groups (Table 3.5), being greatest immediately after feeding and when the diet was hay.

Table 3.5 The percentage of DM in the digesta of HS and LS cows offered hay and pasture (Experiment 3).

Time after feeding (h)	Hay				Pasture			
	HS	LS	sed	sig.	HS	LS	sed	sig.
0	12.04	12.00	0.19	ns	9.10	9.52	0.41	ns
8	10.85	11.34	0.31	ns	8.64	8.95	0.34	ns
21	8.28	8.70	0.44	ns	7.31	7.72	0.46	ns

Experiment 4 Daily DM intakes averaged over the experimental period, excluding measurement days in Period X, were 12.89 and 13.20 kg/cow (sed 0.23, ns) and 144 and 135 g/kg $LW^{0.75}$ (sed 12, ns) for HS and LS cows, respectively. On average, 50% of the DM was consumed during each of the feeding periods. Daily water intakes (consumed in feed and by drinking) averaged 95.4 and 95.2 kg (sed 3.6, ns) for HS and LS cows, respectively, over this period.

On measurement days in Period X, when feed was withheld during the second feeding period, DM intakes were 11% higher for LS cows than for HS cows when expressed per cow (Table 3.6) but were similar when expressed on a metabolic live weight basis (67 and 68 g/kg $LW^{0.75}$, sed 8, ns, for HS and LS cows, respectively). Water intakes were also slightly higher for LS than HS cows, but intakes were similar when expressed per kg of DM consumed (8.2 and 8.1 kg water/kg DM for HS and LS cows, respectively).

Table 3.6 Daily intakes (kg/cow) of DM and water by HS and LS cows on measurement days (Experiment 4).

	HS	LS	sed	sig.
DM	5.99	6.65	0.50	ns
Water	49.4	53.0	3.8	ns

Rather than enhancing the differences between the susceptibility groups observed on pasture in Experiment 3, offering pasture _ad libitum_ to lactating cows resulted in a different pattern of weights and losses of digesta.

LS cows had 6.59 kg (14%) more fluid and 1.51 kg (33%) more DM in the reticulo-rumen than HS cows before the morning feed, 11 h after the last feeding period (Table 3.7). Following feeding, the weights of fluid and DM were 9.40 and 1.85 kg higher for LS than for HS cows (Table 3.7). Calculated losses during feeding were similar for both groups. There was a significant positive relationship between the weight of fluid lost during the feeding period and the weight consumed, but not for DM. The pooled regression coefficient was 0.479 (±0.084, $P<0.001$), with no difference in intercept between the susceptibility groups.

Table 3.7 Quantities (kg) removed from the reticulo-rumen before and after feeding and the calculated net losses (kg) during the feeding period of fluid, DM and total digesta for HS and LS cows offered pasture (Experiment 4).

		HS	LS	sed	sig.
Fluid	Pre-feed	45.47	52.06	2.39	+
	Post-feed	66.06	75.46	4.69	ns
	Loss	26.63	27.44	1.47	ns
DM	Pre-feed	4.63	6.14	0.30	**
	Post-feed	7.83	9.68	0.65	*
	Loss	2.57	2.86	0.30	ns
Total	Pre-feed	50.10	58.20	2.60	*
	Post-feed	73.89	85.14	5.26	+
	Loss	29.20	30.30	1.60	ns

Regression analyses for the relationships between the quantity of digesta in the reticulo-rumen immediately after the feeding period and its subsequent loss, indicated that there were no differences between susceptibility groups in intercepts, at a pooled slope, for fluid, DM or total digesta (Table 3.8). Thus the actual losses were higher for LS cows than HS cows due to their greater quantity of digesta (Table 3.7) rather than to a greater proportion of digesta lost, as occurred on pasture in Experiment 3. The weights of digesta remaining in the reticulo-rumen at 18 h, calculated from Tables 3.7 and 3.8, were 33.43 and 36.77 kg fluid and 2.61 and 3.43 kg DM, for HS and LS cows, respectively.

The DM content of digesta was significantly higher for LS cows than for HS cows before feeding, but not at subsequent sample times (Table 3.9).

Table 3.8 Pooled regression coefficients (b ± standard error) and intercepts for the loss (kg) of fluid, DM and total digesta over 18 h on the quantity (kg) in the reticulo-rumen at 0 h for HS and LS cows offered pasture (Experiment 4).

	b ±	se	sig.	Intercepts HS	 LS	sed	sig.	R^{2a}
Fluid	0.596	0.179	*	-6.74	-6.30	3.52	ns	0.59
DM	0.722	0.076	***	-0.43	-0.74	0.25	ns	0.91
Total	0.597	0.160	*	-6.26	-6.00	3.57	ns	0.65

[a] Percentage of variance accounted for by the regression

Table 3.9 The percentage of DM in the digesta of HS and LS cows offered pasture (Experiment 4).

Sample time	(h)	HS	LS	sed	sig.
Pre-feed		9.21	10.50	0.30	*
Post-feed	0	10.60	11.32	0.37	ns
	18	7.29	8.07	0.63	ns

3.3.4 Rumen fluid dilution rate

Experiment 3 Average dilution rates of about 7 and 5% per h were obtained on hay and pasture, respectively (Table 3.10). There were no differences between susceptibility groups. Data were variable within

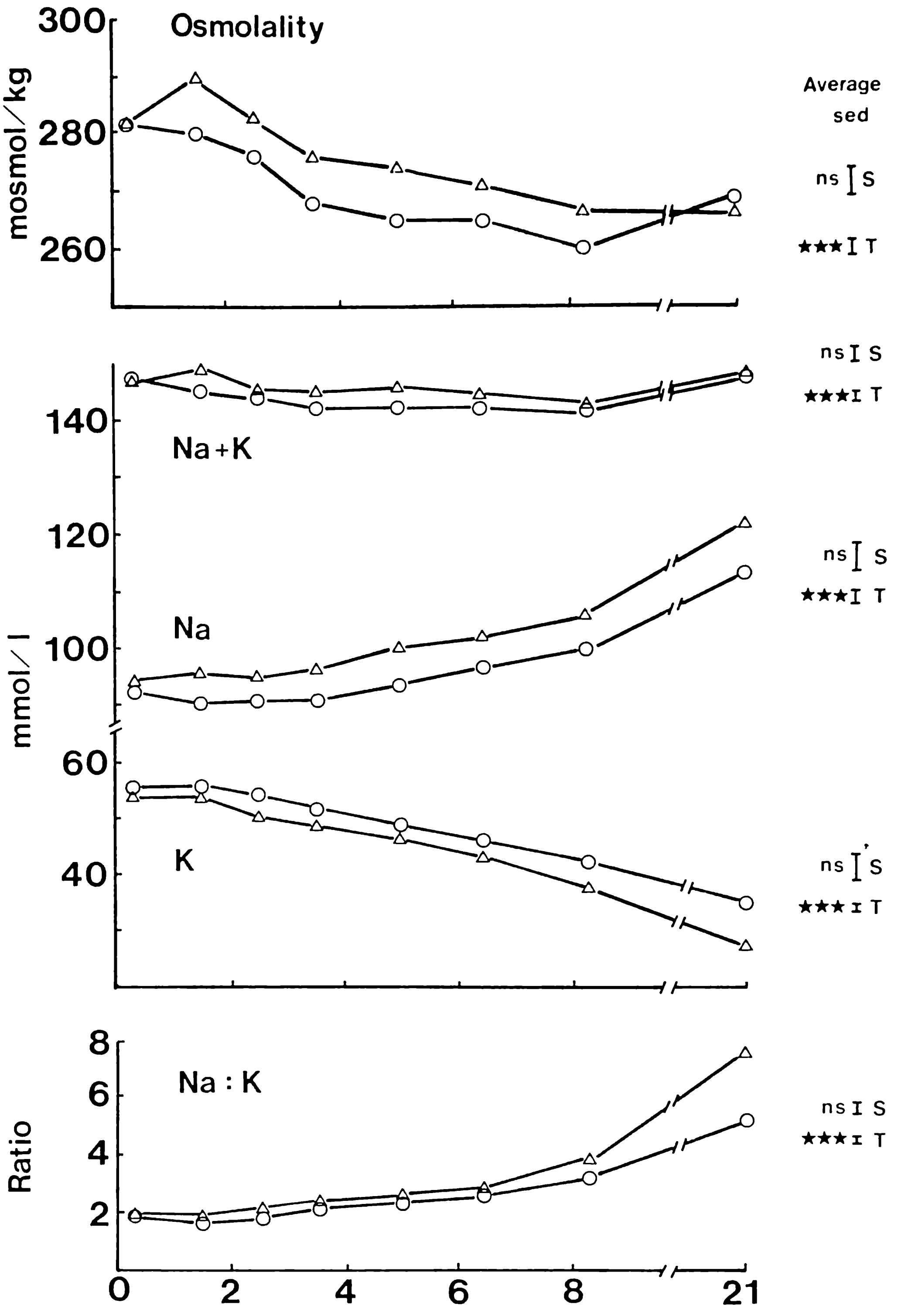


Figure 3.3 Effects of time after feeding on osmolality, concentrations of Na, K, Na + K and Na:K ratio in rumen fluid from non-lactating HS (o) and LS (△) cows offered hay. Sed values are for comparisons between susceptibility groups averaged for all times (S) and between times of sampling averaged for both susceptibility groups (T).

and between cows, suggesting that factors other than susceptibility were major determinants of dilution rate.

The calculated net fluid inflows to the reticulo-rumen over the 8 h post-feeding period were similar for both susceptibility groups (Table 3.10). Outflow of fluid to the omasum was slightly higher for LS cows than for HS cows when offered hay, but values on pasture were similar for both susceptibility groups.

Experiment 4 Average dilution rates over the 7 h post-feeding period were similar, at about 14%, for both susceptibility groups (Table 3.10). The weights of digesta at 7 h were not obtained and therefore the net inflows and the outflows of fluid were not able to be estimated.

Table 3.10 Rumen fluid dilution rates (%/h) and net inflows to the reticulo-rumen and outflows to the omasum (ℓ/h) for HS and LS cows offered hay and pasture.

	Hay				Pasture			
	HS	LS	sed	sig.	HS	LS	sed	sig.
Experiment 3								
Dilution rate	7.06	7.60	0.43	ns	4.98	5.21	0.43	ns
Net inflow	3.51	4.01	0.52	ns	2.46	2.35	0.32	ns
Outflow	4.66	5.64	0.46	+	3.40	3.79	0.58	ns
Experiment 4								
Dilution rate					14.35	13.34	0.88	ns

3.3.5 Composition of digesta

Experiment 3

Hay <u>Osmolality</u> values of rumen fluid (Figure 3.3) were higher for LS than HS cows over the 8 h post-feeding period, but the differences were not significant. There were no interactions between susceptibility group and sample time. Average values were 271 and 277

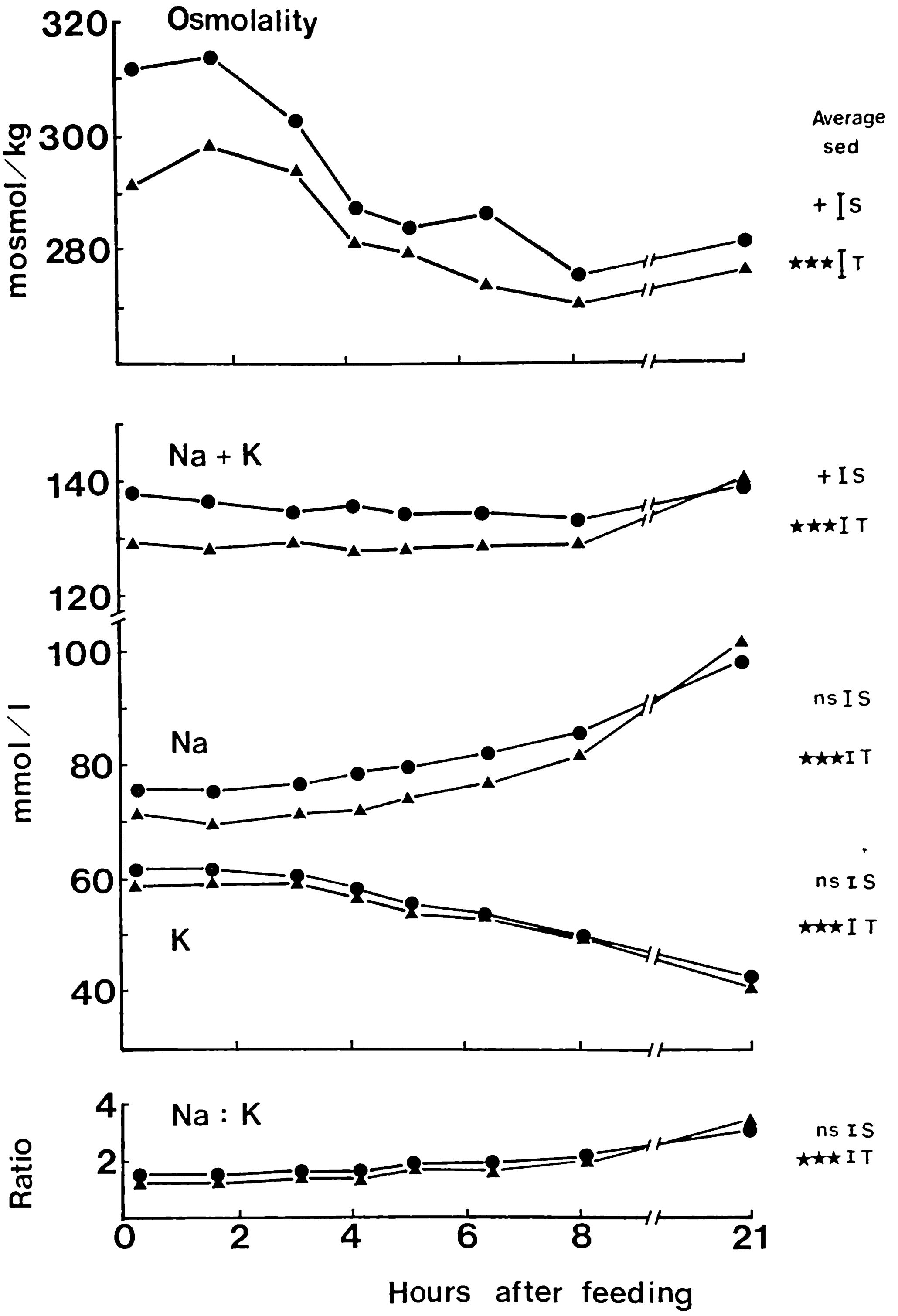


Figure 3.4 Effects of time after feeding on osmolality, concentrations of Na, K, Na + K and Na:K ratio in rumen fluid from non-lactating HS (●) and LS (▲) cows offered pasture. Sed values are for comparisons between susceptibility groups averaged for all times (S) and between times of sampling averaged for both susceptibility groups (T).

mosmol/kg for HS and LS cows respectively. There was no difference between susceptibility groups at 21 h.

Osmolality differed significantly between sample times, reaching a maximum at 1-1.5 h after feeding.

Na and K (Figure 3.3) - LS cows maintained slightly, but not significantly, higher Na and lower K concentrations than HS cows over the 8 hour post-feeding period. There were no interactions between susceptibility group and sample time for Na or K. Average values for Na over 8 h were 93.4 and 97.9 mmol/ℓ for HS and LS cows respectively. Corresponding values for K were 50.1 and 47.4 mmol/ℓ. There were no significant differences between susceptibility groups at 21 h. Values differed significantly between sample times, with the concentration of Na increasing and that of K decreasing with time after feeding.

The Na + K concentration averaged 143 and 145 mmol/ℓ for HS and LS cows, respectively, over the 8 h post-feeding period. The concentration gradually declined over 8 h after feeding.

The Na:K ratio increased with time after feeding, and no differences were observed between susceptibility groups.

Pasture Osmolality - In contrast to data obtained on hay, LS cows maintained lower osmolality values than HS cows during the post-feeding period, significant at 0-2 h and at 7-8 h (Figure 3.4). Average values over the 8 h were 295 and 285 mosmol/kg ($P<0.10$) for HS and LS cows, respectively. Values were similar for both susceptibility groups at 21 h.

Osmolality values differed significantly between sample times, reaching a maximum at 1.5-2 h after feeding.

Na and K - LS cows maintained a slightly, but not significantly, lower concentration of Na and a similar concentration of K, to those of HS cows. Values for Na over the 8 h averaged 79.0 and 73.5 mmol/ℓ for HS and LS cows, respectively. Corresponding values for K were 56.8 and 55.6 mmol/ℓ. The concentration of Na was lowest, and that of K highest, 1-2 h after feeding, with significant differences between sample times. There were no differences between susceptibility groups in concentrations of Na or K at 21 h.

The Na + K concentration declined after feeding and was significantly lower for LS cows than for HS cows at 0-2, 4, 5, and 7 h after feeding. Values averaged 136 and 129 mmol/ℓ ($P<0.10$) for HS and LS cows respectively over the 8 h period. There was no difference between susceptibility groups at 21 h.

The Na:K ratio increased with time after feeding and no differences existed between susceptibility groups.

The concentrations of Na and Na + K were lower, and that of K higher, on pasture compared to hay. Osmolality values were higher on pasture than on hay.

Rumen fluid Na concentration and water intake The relationships between the quantity of water consumed by drinking and the osmolality and Na and K concentrations of rumen fluid were examined for both diets (Table 3.11). The regression coefficients were significant for osmolality and Na concentration, but not for K concentration, immediately after feeding and when averaged over 8 h after feeding. Increases in water intake were associated with decreases in osmolality and concentration of Na. There were also significant negative relationships between water intake and osmolality at 21 h on hay, and between water intake and Na concentration at 21 h on pasture. A positive relationship existed between water intake and the concentration of K at 21 h on pasture.

Table 3.11 Regression coefficients (b ± standard error) for the relationships between the quantity of water consumed by drinking and the osmolality and concentrations of Na and K in rumen fluid.

	Time after feeding (h)								
	0			0-8			21		
	b	± se	sig.	b	± se	sig.	b	± se	sig.
Hay									
Osmolality	-1.97	0.39	***	-1.68	0.42	***	-0.96	0.40	*
Na	-1.38	0.34	***	-0.84	0.31	*	-0.06	0.43	ns
K	0.10	0.41	ns	0.07	0.41	ns	-0.36	0.37	ns
Pasture									
Osmolality	-2.86	0.30	***	-2.23	0.21	***	-0.19	0.16	ns
Na	-1.18	0.37	**	-1.13	0.36	**	-1.12	0.47	*
K	-0.40	0.24	ns	-0.12	0.28	ns	0.83	0.43	+

Effect of NaCl supplementation The analysis of saliva samples indicated that the Na status of the cows was low in weeks 1 and 2 on pasture, but this improved in week 3 following 5 days of supplementation with NaCl (Appendix 3a).

Supplementation increased the concentrations of Na and Na + K and decreased the concentration of K in rumen fluid (Appendix 3b), evident when averaged over the 8 h post-feeding period, or at 21 h. Supplementation restored concentrations of Na and K to values similar to those observed on hay. On pasture there were significant interactions between susceptibility group and week in the concentrations of Na, K and Na + K. LS cows had a lower concentration of Na than HS cows in weeks 2 and 3, compared to week 1, and a higher concentration of K than HS cows in week 3 compared to weeks 1 and 2.

There were also differences between weeks 1, 2 and 3 on pasture in intake and in the quantity of digesta (Appendix 3c), although they cannot be directly associated with Na status. Whereas on hay the intakes of fluid and DM and their quantities in the rumen were

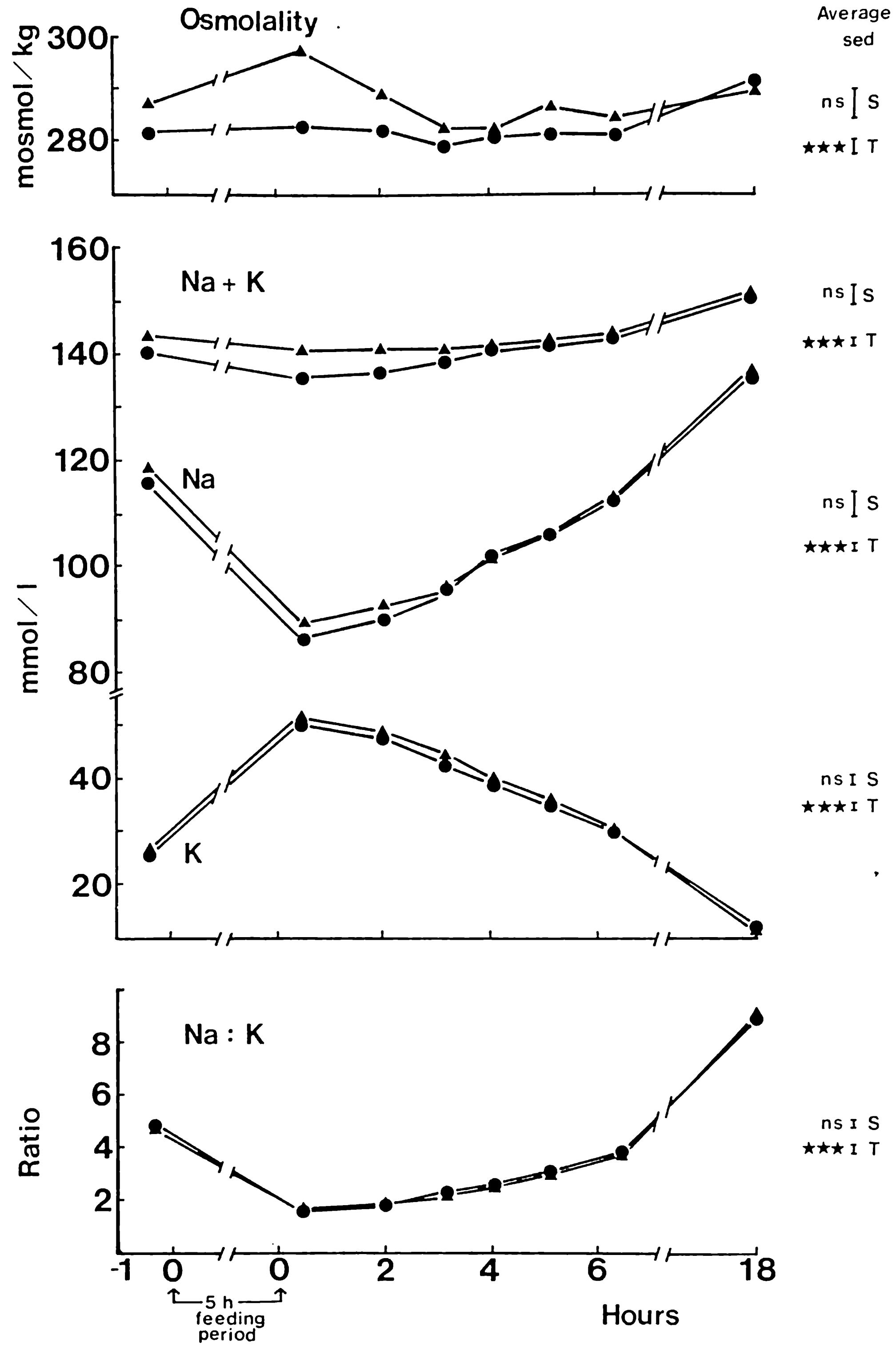


Figure 3.5 Effects of time relative to feeding on osmolality, concentrations of Na, K, Na + K and Na:K ratio in rumen fluid from lactating HS (●) and LS (▲) cows offered pasture. Sed values are for comparisons between susceptibility groups averaged for all times (S) and between times of sampling averaged for both susceptibility groups (T).

relatively constant, on pasture there was a significant difference between the weeks in the quantity of fluid and DM digesta lost over 21 h, and this appeared to be due to lower intakes and, as a consequence, a lower quantity of digesta in the rumen immediately after feeding. The quantity of digesta in the rumen 21 h after feeding was constant across all weeks. There were no interactions between week and susceptibility group.

The declining intake with time of pasture DM on measurement days reflected the general trend that occurred over the experimental period. Intakes of water were more variable and showed no trends.

Experiment 4 Osmolality of rumen fluid sampled before feeding averaged 282 and 287 mosmol/kg for HS and LS cows, respectively (Figure 3.5). Average values over 7 h after feeding were lower for HS than LS cows, averaging 281 and 287 mosmol/kg, respectively, but the differences were not significant. Values were significantly different between sample times. Values averaged 291 and 290 mosmol/kg for HS and LS cows, respectively, at 18 h.

Na, K and Na + K concentrations and the Na:K ratio (Figure 3.5) were similar for both susceptibility groups, at all sample times. The concentration of Na increased and that of K decreased during the post-feeding period, as occurred in Experiment 3. Concentrations were significantly different between sample times for Na, K, Na + K and Na:K ratio. Average concentrations over 7 h after feeding for HS and LS cows, respectively, were 98.7 and 99.6 mmol/ℓ for Na and 40.6 and 42.1 mmol/ℓ for K.

pH was higher in digesta in the reticulum than in the rumen (Table 3.12), and increased with time after feeding in both regions (sed 0.04, $P<0.001$).

Table 3.13 Water-filled reticulo-rumen capacity and digesta quantity expressed as a proportion of capacity for HS and LS cows.

Experiment 3

Time of estimate	Hay		Pasture					
	Pre-feed		Pre-feed		Post-feed			
	HS	LS	HS	LS	HS	LS	sed	sig.
Capacity (kg)	83.9	92.9	83.3	95.0	84.9	92.7	6.1	ns

Fill (%)	Hay				Pasture			
	HS	LS	sed	sig.	HS	LS	sed	sig.
0 h	79.3	76.3	5.5	ns	73.2	66.2	6.1	ns
21 h	48.6	43.9	3.1	ns	47.5	33.3	3.3	**

Experiment 4

	HS	LS	sed	sig.
Capacity (kg)	108.9	127.9	6.8	*
Fill (%)				
Pre-feed	46.9	45.7	3.9	ns
Post-feed	69.2	67.1	7.9	ns

LS cows maintained slightly lower pH values than HS cows, significant at 7 and 18 h. There was no interaction between site and susceptibility group.

Table 3.12 pH of digesta in the reticulum (Re) and rumen (Ru) of HS and LS cows before and after offering pasture.

Sample time	(h)	Site Re	Ru	sed	sig.	Susceptibility HS	LS	sed	sig.
Pre-feed		6.76	6.66	0.02	***	6.76	6.66	0.07	ns
Post-feed	0	6.36	6.21	0.03	***	6.37	6.20	0.13	ns
	3	6.45	6.17	0.03	***	6.38	6.24	0.08	ns
	5	6.51	6.36	0.04	**	6.51	6.36	0.09	ns
	7	6.77	6.52	0.04	**	6.72	6.56	0.04	**
	18	7.25	7.16	0.03	*	7.27	7.14	0.06	*

3.3.6 Reticulo-rumen capacity

Experiment 3 Estimates of capacity before feeding were similar for both diets (Table 3.13), suggesting that the combination of 6 weeks advance in pregnancy and a change in diet from hay to pasture did not affect capacity. Capacity was unaffected by feeding as estimates were similar before and after offering pasture.

Capacity was 9.5 kg (sed 6.0, ns) larger for LS than HS cows, but was 22% of live weight for both susceptibility groups. The condition of the cow affected capacity when capacity was expressed as a proportion of live weight. Capacity of 4 fat cows (condition score (CS) 7.3 to 8.8) and 6 thinner cows (CS 4.3 to 5.0) was 16 and 25% of live weight, respectively. HS and LS cows did not differ in average CS.

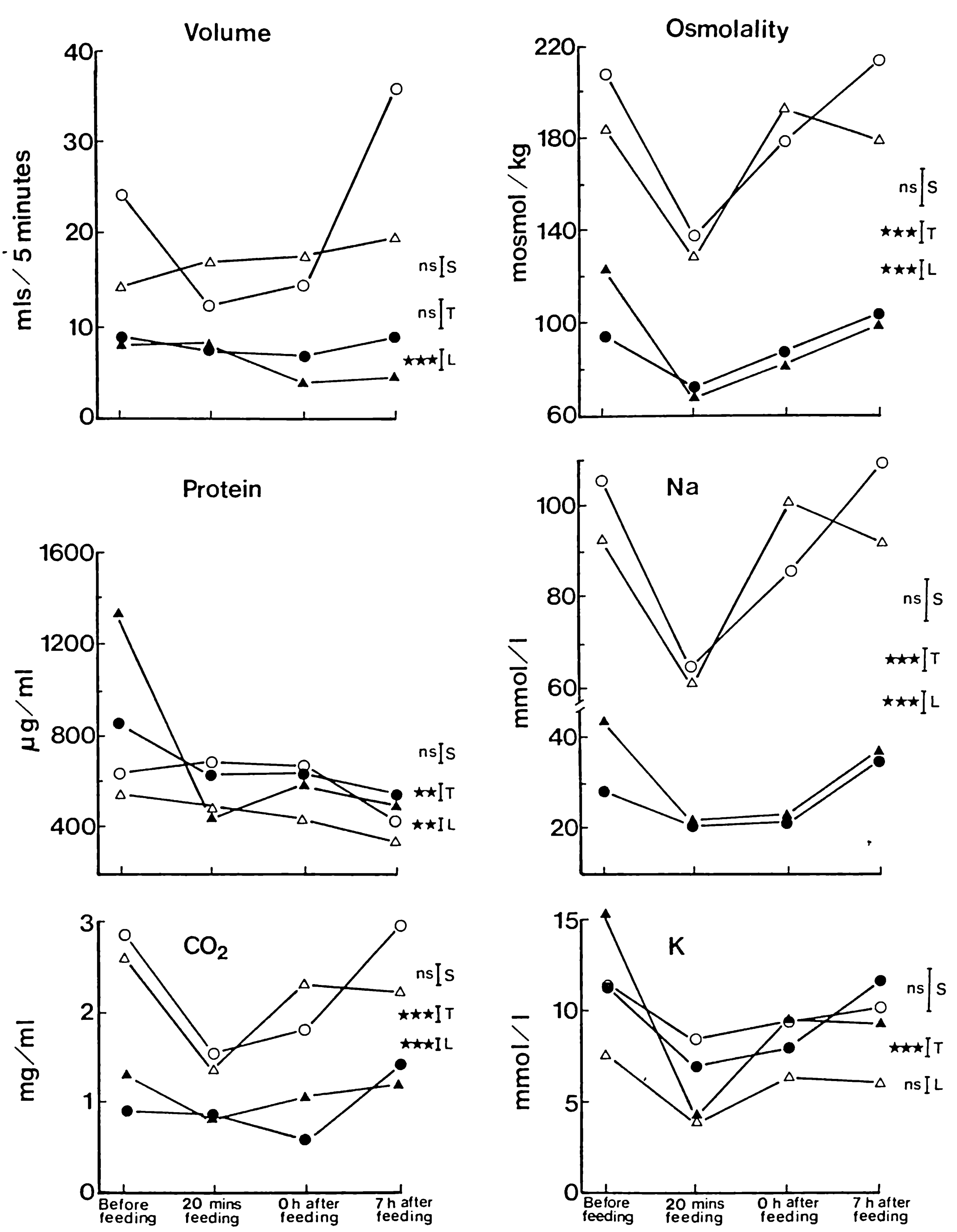


Figure 3.6 The volume, osmolality and concentrations of protein, CO_2, Na and K in saliva collected from the front (●,▲) and rear (○,△) of the mouth of HS (●,○) and LS (▲,△) cows before feeding, after 15-25 minutes of feeding and at 0 and 7 h after the feeding period ceased. Sed values are for comparisons between susceptibility groups averaged for all times and sites (S), between times of sampling averaged for susceptibility groups and sites (T) and between sites of sampling averaged for susceptibility groups and times (L).

Estimates of digesta fill were similar for both susceptibility groups at 0 h on both diets and at 21 h when offered hay, but fill was significantly less for LS cows than for HS cows at 21 h when offered pasture (Table 3.13).

There was no difference between hay and pasture or between susceptibility groups in the relationship between the weight of digesta and its volume. The data for digesta removed immediately after feeding are shown in Appendix 4.

Experiment 4 Capacity was 19 kg greater ($P<0.05$) for LS cows than for HS cows when lactating (Table 3.13), but was 28% of live weight for both susceptibility groups. Condition score ranged from 3.5 to 5.9 and there was no effect of CS on capacity as a proportion of live weight. Digesta fill before and after feeding was similar for both susceptibility groups.

The capacity of lactating cows was 34% greater (range 19 to 67%) than that of non-lactating, pregnant cows.

3.3.7 **Composition of saliva and blood** (Experiment 4)

Saliva

Volume Similar volumes of saliva from the front and rear of the mouth were collected in 5 minutes from HS and LS cows at each sample time, except that LS cows produced less front ($P<0.05$) and rear ($P<0.10$) saliva 7 h after the feeding period (Figure 3.6). A significantly greater volume of saliva was obtained from the rear than from the front collection at all sample times. There was no difference in volume between sample times.

Osmolality, Na, and K There were no differences between susceptibility groups in osmolality or Na concentration of either front or rear saliva (Figure 3.6). K concentration was higher in rear saliva from HS cows than from LS cows ($P<0.001$) at all sample times,

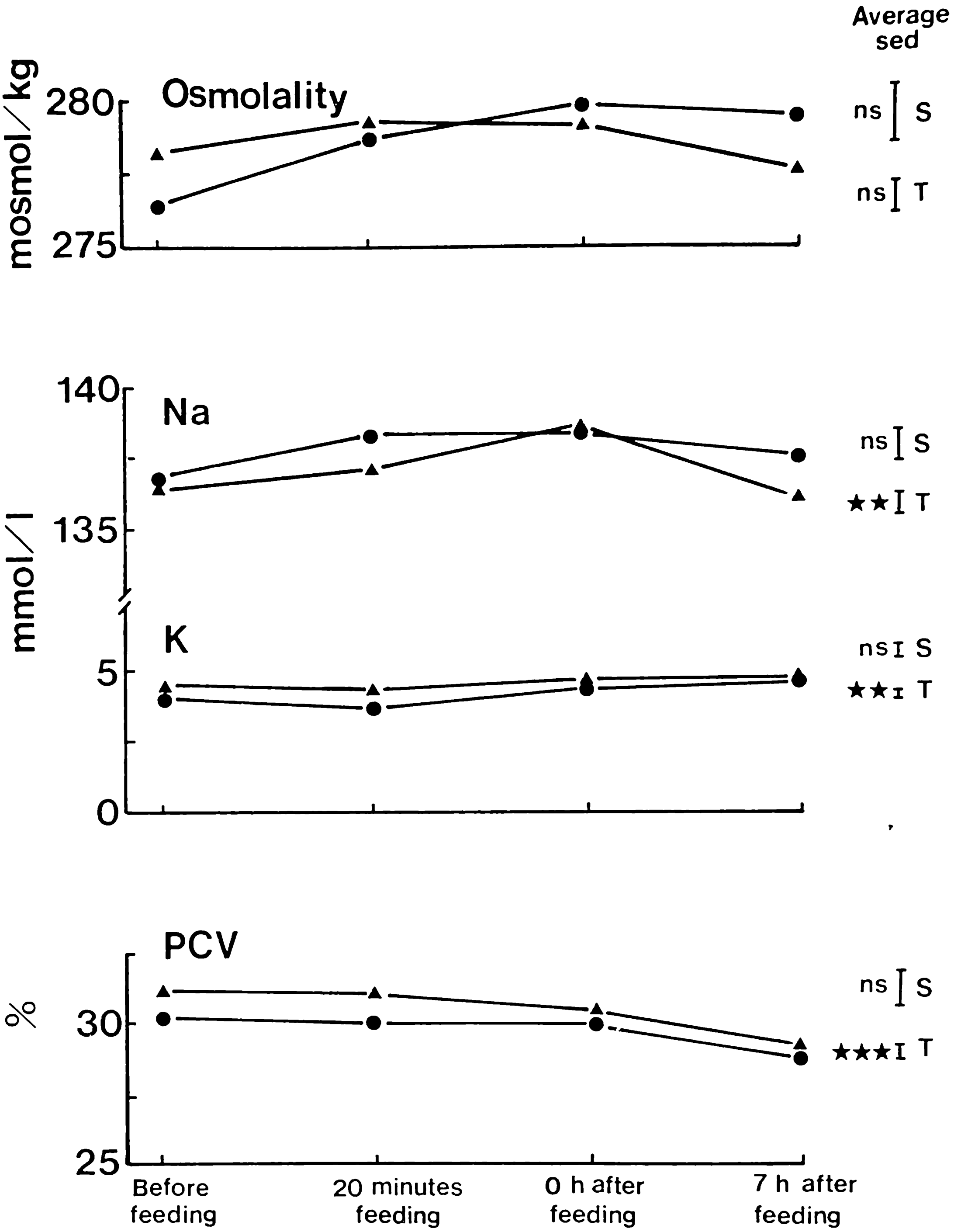


Figure 3.7 Osmolality and concentrations of Na and K in plasma and packed cell volume (PCV) of blood from HS (●) and LS (▲) cows before feeding, after 15-25 minutes of feeding and at 0 and 7 h after the feeding period ceased. Sed values are for comparisons between susceptibility groups averaged for all times (S) and between times of sampling averaged for both susceptibility groups (T).

and there was an interaction ($P<0.05$) between susceptibility group and sample time in K concentration of front saliva (Figure 3.6).

Rear saliva was higher in osmolality and Na concentration than was front saliva, but was similar in K concentration. Osmolality and concentrations of Na and K were significantly lower after 20 minutes of feeding than they were at other times, for both front and rear saliva.

Protein With the exception of front saliva before feeding, the protein concentration (Figure 3.6) of saliva from HS cows tended to be higher than that of LS cows; this was significant ($P<0.10$) for rear collections after 20 minutes of feeding and at the end of the feeding period. The concentration of protein was higher in front than rear saliva when averaged for all times ($P<0.01$) and was higher in front saliva before feeding than at the other sample times ($P<0.01$).

CO_2 The concentration of CO_2 (Figure 3.6) was not determined on 18 of the 80 samples due to insufficient sample. There were no differences between susceptibility groups in concentration of CO_2. Rear saliva was higher in concentration of CO_2 than front saliva at all sample times ($P<0.001$) and the concentration was lower after 20 minutes of feeding than at other times ($P<0.001$).

Blood

Packed cell volume (PCV) PCV was similar for both susceptibility groups at each sample time, and was lower 7 h after feeding ($P<0.001$) than at the other sample times (Figure 3.7).

Plasma osmolality, Na and K Osmolality of plasma was similar for both susceptibility groups and did not differ between sample times (Figure 3.7). Na concentration was higher at the end of the feeding period than either before feeding or 7 h after the feeding period ($P<0.01$) but there were no differences between susceptibility groups.

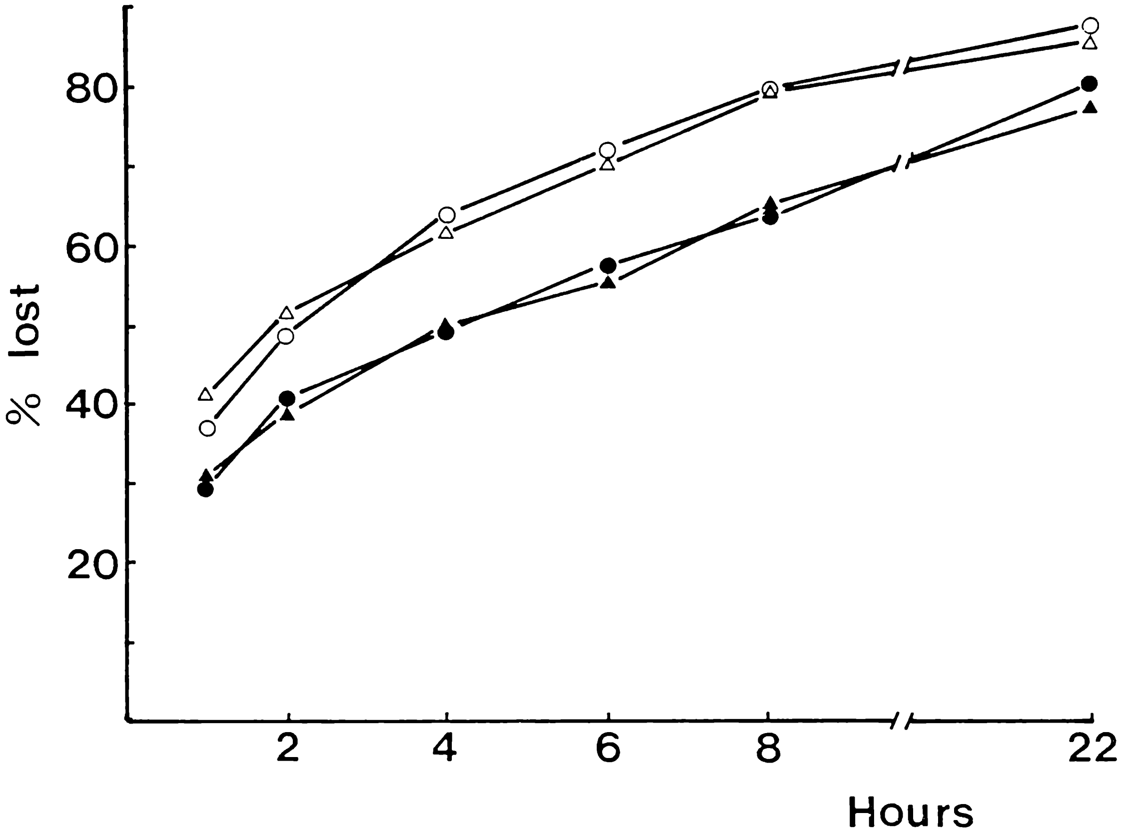


Figure 3.8 The percentage of DM (●,▲) and nitrogen (○,△) lost from food + saliva incubated in nylon bags in the ventral rumen of HS (●,○) and LS (▲,△) cows. Pasture was offered from 0-4 h and 7-11 h after placement of bags at 0 h.

K concentration was lower after 20 minutes of feeding than at other sample times ($P<0.01$), and was lower for HS than LS cows ($P<0.05$) after 20 minutes of feeding, but not at the other times.

3.3.8 Bolus collection and incubation in nylon bags

Boli The quantity of saliva in the bolus material collected at the cardia (Table 3.14) was slightly, but not significantly, higher for HS cows than for LS cows. Concentrations of minerals (Table 3.15) were similar for both susceptibility groups. Comparison of food and bolus mineral composition indicated the extent, particularly for Na, of salivary modification of food mineral content.

DM and nitrogen degradation There were no differences between susceptibility groups in the rate or extent of loss of DM or nitrogen from the ingesta (food + saliva) incubated in the rumen for 22 h (Figure 3.8).

Table 3.14 Quantity of saliva in boli collected at the cardia of HS and LS cows offered pasture.

	HS	se	LS	se
kg/kg DM	4.26	0.30	4.09	0.22
kg/minute	0.19	0.02	0.16	0.03

Table 3.16 A summary of results obtained in Experiments 3 and 4 indicating whether values for LS cows were greater than (+), similar to (=) or less than (-) those for HS cows.

			Experiment 3		Experiment 4
			Hay	Pasture	Pasture
Live weight (LW)			+	+	+
DM intake			=	=	=
Water intake			=	+	=
Digesta	pre feed	kg			+
		fill %			=
	0 h post feed	kg	=	=	=(+)[a]
		fill %	=	=	=
	18 or 21 h post-feed	kg	=	-	=
		fill %	=	-	=
	proportion lost		=	+	=
	DM%		=	=	(+)
Rumen capacity		% LW	=	=	=
Rumen fluid		dilution	=	=	=
		osmol	=(+)	(-)[b]	=(+)
		Na + K	=	(-)	=
		pH			=(-)
DM degradation rate					=
Saliva		osmol			=
		Na			=
		K			(-)
		protein			(-)
		CO_2			=
Blood		PCV			=
Plasma		osmol			=
		Na			=
		K			(+)

[a] =(+) or =(-) - no significant difference between HS and LS cows but trend for LS to be greater (=(+)) or less (=(-)) than HS

[b] (-) or (+) - significant for some sample times or sites of sampling

Table 3.15 Concentrations of minerals (g/100 g DM) in food and in boli collected at the cardia for HS and LS cows offered pasture.

	Boli					
Mineral	HS	se[a]	LS	se[a]	Food	se[b]
Ca	0.53	0.02	0.55	0.02	0.77	0.08
Mg	0.19	0.01	0.18	0.01	0.27	0.01
Na	1.68	0.09	1.68	0.09	0.20	0.01
K	3.04	0.11	3.06	0.06	3.89	0.25
P	0.43	0.02	0.44	0.01	0.45	0.06

[a] se for mean of 5 cows
[b] se for mean of 2 samples

3.4 DISCUSSION

The differences between HS and LS cows in the quantity of digesta and the pattern of loss of digesta from the reticulo-rumen differed for the two experiments (Table 3.16).

The data obtained in Experiment 3 supported, in part, the hypothesis of Cockrem *et al.* (1983) in that the quantities of both fluid and DM in the reticulo-rumen at 21 h were lower for LS than for HS cows when offered pasture, but not when offered hay. In contrast, there were no differences, after adjustment for live weight, between the susceptibility groups in the quantity of digesta in the reticulo-rumen before or after feeding in Experiment 4. Thus these data were inconsistent with the previous experiment and with the hypothesis, but the experiments differed in terms of the level of feeding and feeding routine, timing of measurements relative to feeding, and physiological state of the cows.

The quantity of digesta in LS cows at 21 h when offered pasture in Experiment 3 was less than that of HS cows on that diet, and that of both HS and LS cows offered hay. Fluid intakes when offered hay in Experiment 3 were related to DM intake, as occurred when offered hay and pasture in Experiment 1. When offered pasture in Experiment 3, however, LS cows drank more water than HS cows despite similar DM intakes. The lower DM intakes when offered pasture compared to hay may have been expected to result in decreased quantities of digesta in the rumen both before and after feeding (Ulyatt _et al_. 1984). The question arises as to whether the higher intake of water by LS cows offered pasture was associated with their lower quantity of digesta at 21 h, compared to HS cows. While water intakes are closely correlated with DM intake, the quantity of water consumed per kg DM intake can vary for different diets (Forbes 1968; Warner & Stacy 1972a), and it was not possible to establish whether LS cows increased or HS cows decreased their water intake for the pasture diet. The data suggested that factors other than DM intake affected the water intake of HS or LS cows when offered pasture in Experiment 3. Comparison of week 3 with weeks 1 and 2 on pasture (Appendix 3c) indicated that changes in water intake were more closely related to the quantity of digesta at 0 h, than to the quantity at 21 h, suggesting that the difference between HS and LS cows at 21 h was maintained whether LS cows drank similar or greater amounts of water than HS cows.

LS cows lost a greater quantity of both fluid and DM after feeding than HS cows. Two factors were identified which affected the quantity of digesta lost:

(1) The quantity of digesta present in the reticulo-rumen after feeding. This quantity was the sum of the pre-feed quantity and the inputs (intake, saliva, net water flux across the rumen wall), and outputs (losses to the omasum, absorption of solutes and products of fermentation).

(2) The proportion of the digesta present that was subsequently lost. This depended on the relationship between intake and the quantity of digesta in the reticulo-rumen, since net changes in fluid and DM over 24 h (feeding + post-feeding periods) must have approximated their respective intakes if the cows were in steady state.

Losses were higher for LS than for HS cows when offered hay, due to a combination of a greater quantity of digesta in the rumen (see Table 3.3) and a greater proportional loss (see Table 3.4). Although neither was significant the effects were additive, but they did not significantly alter the quantity of digesta at 21 h of LS cows relative to HS cows. When offered pasture in Experiment 4, a greater quantity of digesta in the reticulo-rumen of LS cows contributed to a greater post-feeding loss. In contrast to these results, the difference in loss between HS and LS cows when offered pasture in Experiment 3 was associated only with a difference in the proportion lost, for both fluid and DM. The greater water intake by LS cows accounted for or contributed to their greater post-feeding fluid loss, but did not explain why the higher intake was associated with a decreased quantity of digesta at 21 h.

The difference in net fluid loss after feeding, over the first 8 h at least, was not due to differences in net fluid input. The calculation of net inflow using the equations of Reid (1965) assumes that both the rate of inflow and the rate of outflow are constant over the period of measurement. Warner & Stacy (1968a) suggested that this was unlikely when there were large changes in volume, as occurred in the present experiments. However, calculation of net inflow using an alternative equation (Warner & Stacy 1968a) which assumes that the dilution rate is constant and that volume varies at a constant rate, did not significantly alter the relative (HS-LS) net inflows as calculated above.

The greater loss of DM after feeding, and the lower quantity of DM remaining at 21 h, for LS cows than for HS cows offered pasture (Experiment 3) suggests a faster rate of digestion and/or an increased outflow of DM to the omasum in LS cows. Lactating cows did not differ in rate of digestion when it was estimated from the loss of DM from nylon bags. The rate of digestion of chewed material has been shown to be higher than that for unchewed (Poppi _et al_. 1981a), therefore differences that can occur between animals in chewing efficiency (Lee & Pearce 1984) might have been reflected in digestion rate, had they existed. The lack of a difference in rate of digestion in Experiment 4 does not preclude the existence of a difference between non-lactating HS and LS cows in Experiment 3, since the two experiments differed in other respects.

A relatively greater loss of DM after feeding for LS cows implies a lower loss during feeding, if total losses over 24 h for HS and LS cows approximate intakes, which were similar. In lactating cows the loss of fluid, but not DM, during feeding increased with increasing intake. This relationship has been previously reported for DM losses (Campling _et al_. 1961); the range of DM intakes in Experiment 4 may have been insufficient to show this relationship.

Associated with the interaction between diet and susceptibility group in losses of digesta and in the quantities of digesta at 21 h in Experiment 3 were differences in osmolality and the concentration of Na + K in rumen fluid after feeding. LS cows maintained a higher osmolality and concentration of Na when offered hay and lower values when offered pasture than did HS cows over the 8 h post-feeding period, although values were similar at 21 h. The lower values when offered pasture may have resulted from dilution due to the extra water consumed, as indicated by the negative correlation between drinking water intake and Na concentration after feeding. If the difference in intake on pasture (6.9 kg) is subtracted from the quantity of fluid removed for LS cows and the concentrations of Na recalculated, the values are similar for both susceptibility groups. However, there was no correlation between water intake and K concentration. Whether the difference in concentration of Na was directly associated with the difference in the quantity of digesta at 21 h cannot be resolved from the data, but is further investigated in Chapters 5 and 6.

The comparison between pasture and hay in Experiment 3 is confounded by the Na status of the cows. The cows were in a marginal to low Na status on both hay and pasture, but particularly on the latter, as indicated by the concentrations of Na and K in rumen fluid and the Na:K ratio in saliva (Denton 1956; Bailey 1961b; Towers 1983). There is also the possibility that the difference between susceptibility groups in the quantity of digesta when offered pasture was associated with the change in Na and K intake, both in quantity and in the K:Na ratio, which occurred when changing from hay to pasture. This involved a large decrease in Na intake, and a small decrease in K intake (due to the decrease in DM intake offsetting the increase in K concentration of the diet). The effects on digesta of changing the intakes of Na and K are investigated and discussed in Chapter 6.

The trends in composition of rumen fluid of lactating cows were similar to those observed on hay, rather than on pasture, for non-lactating cows in Experiment 3. The pH data, indicating no or small differences between HS and LS cows, were similar to those obtained by Mendel & Boda (1961).

There have been few attempts to describe the composition of blood and saliva from HS and LS cows, apart from the salivary protein characteristics (Clarke _et al_. 1974; McIntosh 1975, 1978; McIntosh & Cockrem 1977). Of the salivary parameters measured, HS and LS cows differed only in the concentration of K in saliva from the rear of the mouth, the concentration being higher in HS than LS cows. Mendel & Boda (1961) found a similar result for mixed saliva collected at the cardia in one of two trials. The biological significance of the difference in concentration of K is not clear. It did not apparently influence concentration of K in rumen fluid, although it may have been associated with the lower concentration of K in plasma of HS cows than LS cows 20 minutes after the start of feeding.

The volume and composition of saliva depended on the site of collection, presumably because a greater proportion of parotid saliva was sampled in the collection at the rear of the mouth and mandibular (and possibly sublingual) in the collection at the front of the mouth (Phillipson & Mangan 1959; Kay 1966). Parotid saliva is lower in concentration of protein than mandibular saliva (Phillipson & Mangan 1959), and this was suggested here. Comparison of the saliva data with published data indicates that the collection technique may have been unsatisfactory in measuring composition of saliva during feeding. The volume and concentration of protein increase in both parotid and mandibular secretions at the start of feeding (Brightling _et al_. 1977; Carr & Titchen 1978; Patterson _et al_. 1982; Carr 1984). However, the samples taken 15-25 minutes after the start of feeding were of lower volume and concentration of protein than were those taken before feeding. This was probably caused by the effect of interruption of feeding required to take the sample (Patterson _et al_. 1982). Alternatively, the time of sampling may have been after the peak in saliva flow as the timing of peak parotid flow in sheep has varied from 1 to 20 minutes after feeding (Carr & Titchen 1978; Patterson _et al_. 1982), or the pasture diet may have provided insufficient stimulus for the volume and protein changes to occur. The weights of saliva collected in the boli and data obtained by Carr (1984) suggest the latter was unlikely. The weights of saliva collected in boli at the cardia during 15 minutes of feeding gave calculated flows of 160-190 g/minute, which were similar to other reported estimates for salivary flow in cows offered fresh grass and lucerne diets (Bailey 1961a; Mendel & Boda 1961). The quantities of saliva collected per minute by the mouth bit were less than the total produced. Collection of saliva using a bit is not quantitative and the technique may also suffer from blockage of the tubing by food particles. Thus the volumes estimated using the bit may have been misleading, although they have been used to indicate differences in saliva production in previous studies on HS and LS cows (McIntosh 1975; McIntosh & Cockrem 1977).

Cockrem _et al_. (1983) reported that LS cows had higher PCV values, indicating a lower plasma volume (Ulrych 1973), than HS cows. No evidence of this was found, nor did HS and LS cows differ in osmolality or concentration of Na in plasma at any sample time, or in concentration of K at 3 of the 4 sample times. Feeding had only minor effects on PCV, Na concentration and osmolality, as has been reported for sheep fed at hourly intervals (Dooley & Williams 1975) but not once daily (Warner & Stacy 1965; Stacy & Warner 1966; Christopherson & Webster 1972; Carr & Titchen 1978). The apparent lack of response to feeding may have been due in part to the sampling site, since Carr & Titchen (1978) found that the change in osmolality of portal venous plasma in response to feeding was consistently higher than that from the jugular vein.

The lack of differences between susceptibility groups in the composition of blood and saliva when the cows were lactating in Experiment 4 does not preclude differences having existed when the quantity of digesta differed between susceptibility groups in Experiment 3.

The conflicting results obtained in the two experiments cannot be resolved on the basis of the results reported here. Experiment 3 demonstrated that differences in digesta between HS and LS cows were present in non-lactating cows, at least under some conditions, and work reported in subsequent chapters used the cows in a non-lactating and non-pregnant state. The initial concern arising from Experiment 3 was to establish more precisely the relationship between the quantity of digesta in the reticulo-rumen and subsequent digesta loss.

CHAPTER 4

INVESTIGATION OF SOME FACTORS AFFECTING THE QUANTITY OF DIGESTA IN THE RETICULO-RUMEN

4.1 INTRODUCTION

The experiments reported in this chapter (Experiments 5 and 6) were designed to investigate in more detail the relationships between the quantity of digesta in the reticulo-rumen after feeding, subsequent loss of digesta, and the quantity remaining before the next feeding. Data obtained in Experiments 3 and 4 indicated that losses from the reticulo-rumen after feeding were greater for those cows with a greater quantity of digesta. Data in Experiment 3 suggested that fluctuations in intake, particularly that of water, were associated with changes in the quantity of digesta present after feeding and in subsequent digesta loss, rather than with changes in the quantity remaining at 21 h or before the next feeding. This was investigated in Experiment 5 by manipulating the quantity of digesta in the reticulo-rumen after feeding so that the relationship between quantity and loss could be examined within, rather than between cows.

Subsequently, the data obtained in Experiment 5 on the quantity of digesta at 21 h for HS and LS cows were not consistent with results obtained on pasture in Experiment 3. Following consideration of factors which could have contributed to the differences between experiments, Experiment 6 investigated the effect of maturity of the pasture on the quantity of digesta in the reticulo-rumen.

4.2 MATERIALS AND METHODS

4.2.1 Cows

The cows were those used in Experiments 3 and 4 except that 1 HS cow and 1 LS cow (see Appendix 1) were omitted following diagnosis of rectal lipomastosis (Arbuckle 1962) in October 1983. The cows used in the experiments were not pregnant and had not been lactating for 60 days at the start of Experiment 5.

Cows were housed as described for Experiment 3.

4.2.2 Feeding, experimental design and measurements

Experiment 5 - Manipulation of the quantity of digesta in the reticulo-rumen after feeding

Feeding An irrigated pasture consisting predominantly of perennial ryegrass and white clover was cut daily using a flail harvester and offered at a maintenance feeding level, as described in Chapter 3.2.2. The food was offered for 2 h each day. The fresh weight of food to be offered was estimated daily using a DM content obtained by drying duplicate 100 g samples in a microwave oven. Water was offered to appetite at the end of the feeding period, except on measurement days when water was withheld. Large intakes of drinking water by cows in Experiment 3 had increased the difficulty of returning bailed digesta to the reticulo-rumen after feeding, therefore water was not offered on measurement days in Experiment 5 in order to minimise this problem with any of the treatments.

Fifteen g NaCl was added daily to each cow's food.

The cows were managed in 2 groups of 4 cows (2 HS, 2 LS per group), which differed in feeding and measurements by 1 h. Food was offered at 0700 and 0800 h.

Experimental design The experiment consisted of a 14-day adaptation followed by a 19-day experimental period when 4 treatments were imposed on 4 HS-LS pairs in a Latin square arrangement. At least 3 days were allowed between successive treatments.

The treatments involved inducing differences in the quantity of digesta in the reticulo-rumen immediately after feeding by the addition or removal of digesta, as follows:

Treatment	1	No change (control)
	2	Addition of 10 kg digesta from a cow of the same susceptibility group
	3	Removal of 10 kg digesta
	4	Removal of 15 kg digesta

The treatments were applied to the actual quantity of digesta removed on the measurement day, rather than being adjusted to a set weight. The difference between Treatments 2 and 4 in the weight of digesta returned ranged from 20 to 38 kg for individual cows.

Digesta On each measurement day the digesta was removed from each cow, weighed, sampled for DM content and replaced immediately before feeding, immediately after feeding (0 h) and 7.5 and 21 h later. The treatments were applied to digesta removed at 0 h, after sampling for DM content.

In addition to sampling for DM the digesta of cows on Treatment 1 was sampled at each removal for the analysis of particle size.

Reticulo-rumen capacity Capacity of the reticulo-rumen was estimated, before feeding, on 2 consecutive days at the end of the experimental period.

Live weight Live weights were measured as described in Chapter 3.2.2 for Experiment 3.

Experiment 6 - Restricted feeding of pastures of low, medium and high maturity

Feeding A pasture consisting predominantly of perennial ryegrass, white clover and prairie grass (*Bromus catharticus* Vahl.) was harvested over three 7-day periods at intervals of 2 weeks, in spring 1984. Herbage mass at the start of successive periods was 2500, 4500 and 7500 kg DM/ha, indicating a range from relatively immature to mature feed. These are subsequently referred to as low (L), medium (M) and high (H) maturity.

The pasture was offered for 2 h each day at a maintenance feeding level. The quantity of fresh material to offer was estimated as described for Experiment 5. Water was offered to appetite, but was withheld on measurement days. The cows were managed in 2 groups (2 HS, 2 LS per group) which differed in feeding and measurements by 45 minutes. Food was offered at 1100 and 1145 h.

During the intervals between the experimental periods the cows were grazed on fresh pasture for 2 h each day, with access to a NaCl block.

Digesta On day 7 of each experimental period, digesta in the reticulo-rumen was removed from each cow, weighed, sampled for DM content and replaced immediately before feeding and at 0 and 21 h after feeding.

Live weight Live weights were measured prior to the experiment and at the start and end of the 7-week period, as described in Section 3.2.2 for Experiment 3.

4.2.3 Techniques and sampling procedures

Foods and residues

Daily foods were sampled for DM content and a sample frozen and combined over 2 consecutive 8 and 9 day periods in Experiment 5, or over each of the 7-day periods in Experiment 6. Any residues were weighed and sampled for DM content.

Digesta removal and reticulo-rumen capacity

Techniques for the removal and sampling of digesta and for the estimation of reticulo-rumen capacity were described in Chapter 3.2.3.

Particle size

Samples for the determination of particle size were preserved using formalin at 4% (v/w). The wet sieving procedure was similar to that described by Evans *et al*. (1973), and involved washing a subsample of 20-30 g wet material with about 1.3 ℓ water for 5 minutes through sieves of 4, 2, 1, 0.5 and 0.25 mm square mesh size. The material collected on each sieve was transferred to a tared filter paper, dried at 70 °C for 24 h, and weighed. The water reservoir was drained into a tared container, weighed, and a 100 g sample removed and centrifuged at 2000 rpm for 15 minutes. The pellet was dried and weighed, and referred to as the residue. DM weights of both sieved and residue material were expressed as proportions of the total DM in the sample which was obtained by duplicate DM determinations on the preserved sample. Soluble DM was that unaccounted for on sieves and in the residue and was thus determined by difference.

4.2.4 Analytical procedures

DM, nitrogen, energy, MAD fibre, digestibility *in vitro*, water-soluble sugar and mineral concentrations of freeze-dried food samples and DM content of digesta were determined as described in Chapters 2.2.5 and 3.2.4.

4.2.5 Calculations and statistical analysis

Digesta losses were calculated as described in Chapter 3.2.5, using the appropriate time intervals, which were:

Experiment 5 - Feeding period of 160 minutes
Post-feeding periods of 450 minutes (0-7.5 h) and 830 minutes (7.5-21.3 h; the latter is subsequently referred to as 21 h)

Experiment 6 - Feeding period of 150 minutes
Post-feeding period of 1290 minutes (0-21.5 h)

Regression equations describing the relationships between the quantity of digesta in the reticulo-rumen and its subsequent loss were obtained for each cow in Experiment 5. The slopes of the regression lines were statistically analysed for significance of differences between cows.

Analysis of variance models were used to test parameters in Experiments 5 and 6 for differences between susceptibility groups or between susceptibility groups and pasture maturity.

4.3 RESULTS

4.3.1 **Experiment 5** - Manipulation of the quantity of digesta in the reticulo-rumen after feeding

Diet composition

Composition of the pasture offered is given in Table 4.1.

Table 4.2 Intakes (kg/cow) on measurement days, and the quantities (kg) removed from the reticulo-rumen before and after feeding and calculated net losses (kg) during feeding of fluid, DM and total digesta for HS and LS cows offered pasture (Experiment 5).

		HS	LS	sed	sig.
Fluid					
Intake		29.97	31.03	1.21	ns
Digesta	pre-feed	37.74	33.80	2.34	ns
	post-feed	60.75	61.37	2.51	ns
	loss	3.74	0.46	1.03	*
DM					
Intake		5.01	5.20	0.21	ns
Digesta	pre-feed	3.14	3.50	0.19	ns
	post-feed	7.63	7.95	0.31	ns
	loss	0.26	0.44	0.16	ns
Total					
Intake		34.98	36.23	1.42	ns
Digesta	pre-feed	40.88	37.30	2.46	ns
	post-feed	68.38	69.32	2.77	ns
	loss	4.00	0.90	0.96	*

Table 4.1 Energy content (MJ/kg DM), digestibility *in vitro* (%) and concentrations (g/100 g DM) of MAD fibre, water-soluble sugar, nitrogen (N) and minerals in pasture (Experiment 5).

Variate	Value	se[a]
Energy	19.01	0.18
Digestibility	67.05	0.30
MAD fibre	26.59	1.53
Sugar	3.21	0.33
N	3.77	0.13
Ca	0.96	0.08
Mg	0.28	0.01
Na	0.13	0.02
K	3.39	0.30
P	0.34	0.01

[a] Standard error for the average of 2 samples

Live weight

LS cows were 41 kg heavier than HS cows (423 and 382 kg respectively, sed 17, $P<0.05$), thus maintaining the differences observed in previous experiments with 10 pregnant or lactating cows.

Intake and the quantity of digesta in the reticulo-rumen

Intakes of DM and water were similar for both susceptibility groups (Table 4.2). When expressed on a live weight basis, DM intakes were 58 and 55 g/kg $LW^{0.75}$ (sed 2, ns) for HS and LS cows, respectively. These intakes were 60% higher than those obtained on pasture, and similar to those obtained on hay, in Experiment 3. Water was withheld on measurement days therefore intakes of water could not differ between susceptibility groups unless associated with a difference in DM intake. On non-measurement days the HS and LS cows drank 2.8 and 8.5 kg respectively (sed 2.5, $P<0.10$).

The quantities of digesta removed from the reticulo-rumen before and after feeding, before the treatments were applied, are given in Table 4.2.

Table 4.3 Regression coefficients (b ± standard error) and intercepts for the loss (kg) of fluid, DM and total digesta from the reticulo-rumen during 7.5 and 21 h after feeding on the quantity (kg) present at 0 and 7.5 h for HS and LS cows offered pasture.

	Time after feeding (h)	b	± se	sig.	Intercepts HS	Intercepts LS	sed	sig.	R^{2a}
Fluid									
	0-7.5	0.338	0.042	***	-9.73	-5.19	2.00	+	0.75
	7.5-21	0.460	0.105	***	-8.08	-7.13	1.05	ns	0.47
	0-21	0.621	0.066	***	-13.52	-9.52	1.98	ns	0.81
DM									
	0-7.5	0.387	0.050	***	-0.71	-0.86	0.13	ns	0.73
	7.5-21	0.428	0.065	***	-0.02	0.07	0.13	ns	0.67
	0-21	0.628	0.038	***	-0.43	-0.39	0.06	ns	0.93
Total									
	0-7.5	0.340	0.039	***	-10.22	-5.82	2.07	+	0.79
	7.5-21	0.455	0.097	***	-8.09	-6.99	1.03	ns	0.50
	0-21	0.619	0.059	***	-13.70	-9.80	2.01	ns	0.84

[a] Percentage of variance accounted for by the regression

LS cows had 3.94 kg less fluid in the reticulo-rumen before feeding than did HS cows, which was about half the difference obtained at a comparable time, relative to feeding, when the cows were offered pasture in Experiment 3. HS and LS cows had similar quantities of DM in the reticulo-rumen before feeding.

The quantities of fluid and DM in the reticulo-rumen immediately after feeding were similar for both susceptibility groups. The calculated net loss of fluid over the feeding period was 3.28 kg higher for HS cows than for LS cows.

The regression equations describing the effect of the quantity of digesta in the reticulo-rumen on its subsequent loss for individual cows indicated there were no significant differences in slope between cows within or between susceptibility groups. Data were therefore combined using analysis of covariance in order to obtain the regression equations at an average slope (Table 4.3).

The regression coefficients were similar to or slightly higher than those obtained on pasture in Experiment 3, and were highly significant ($P<0.001$). The values for the intercepts for fluid (and total digesta) were similar in both experiments over 7.5 or 8 h, but not over 8-21 h, and the difference between susceptibility groups in intercepts over 0-21 h in Experiment 5 (4.00 kg, -13.52 vs -9.52, see Table 4.3) was about half that obtained in Experiment 3, and was not significant. There were no differences between susceptibility groups in the intercepts for DM loss, thus over 21 h there were no significant differences between susceptibility groups in the proportions of fluid or DM lost from the reticulo-rumen. Although not significant, the difference between HS and LS cows in intercepts for fluid loss over 0-21 h indicated that LS cows lost 4.0 kg more fluid after feeding than HS cows. HS cows lost 3.28 kg more fluid during feeding than LS cows, therefore losses of fluid over 24 h were similar for both susceptibility groups.

A comparison of the quantities of fluid and DM removed before feeding with those removed at 21 h for the control treatment (Table 4.4) indicated that similar differences existed between susceptibility groups in quantities at both times despite differences in fluid intake in the previous 24 h. LS cows drank 6.3 kg more water than HS cows during the feeding period 24 h prior to the first pre-feed digesta estimate, but there was no drinking water available over the subsequent 24 h. The quantities removed at 21 h shown in Table 4.4 include an adjustment for the weights of fluid and DM removed or lost during sampling at 0 h and at 7.5 h, but no adjustment has been made for losses during the pre-feed digesta removal.

Table 4.4 The quantities (kg) of fluid and DM in the reticulo-rumen before feeding and at 21 h after feeding for HS and LS cows offered pasture (Experiment 5).

	Relative to feeding	HS	LS
Fluid	Pre-feed	37.03	34.39
	21 h post-feed	35.85	32.74
DM	Pre-feed	3.17	3.59
	21 h post-feed	3.25	3.42

LS cows maintained a slightly higher digesta DM content than HS cows; this difference was significant before feeding and at 7.5 h (Table 4.5). There was no effect of treatment on digesta DM content.

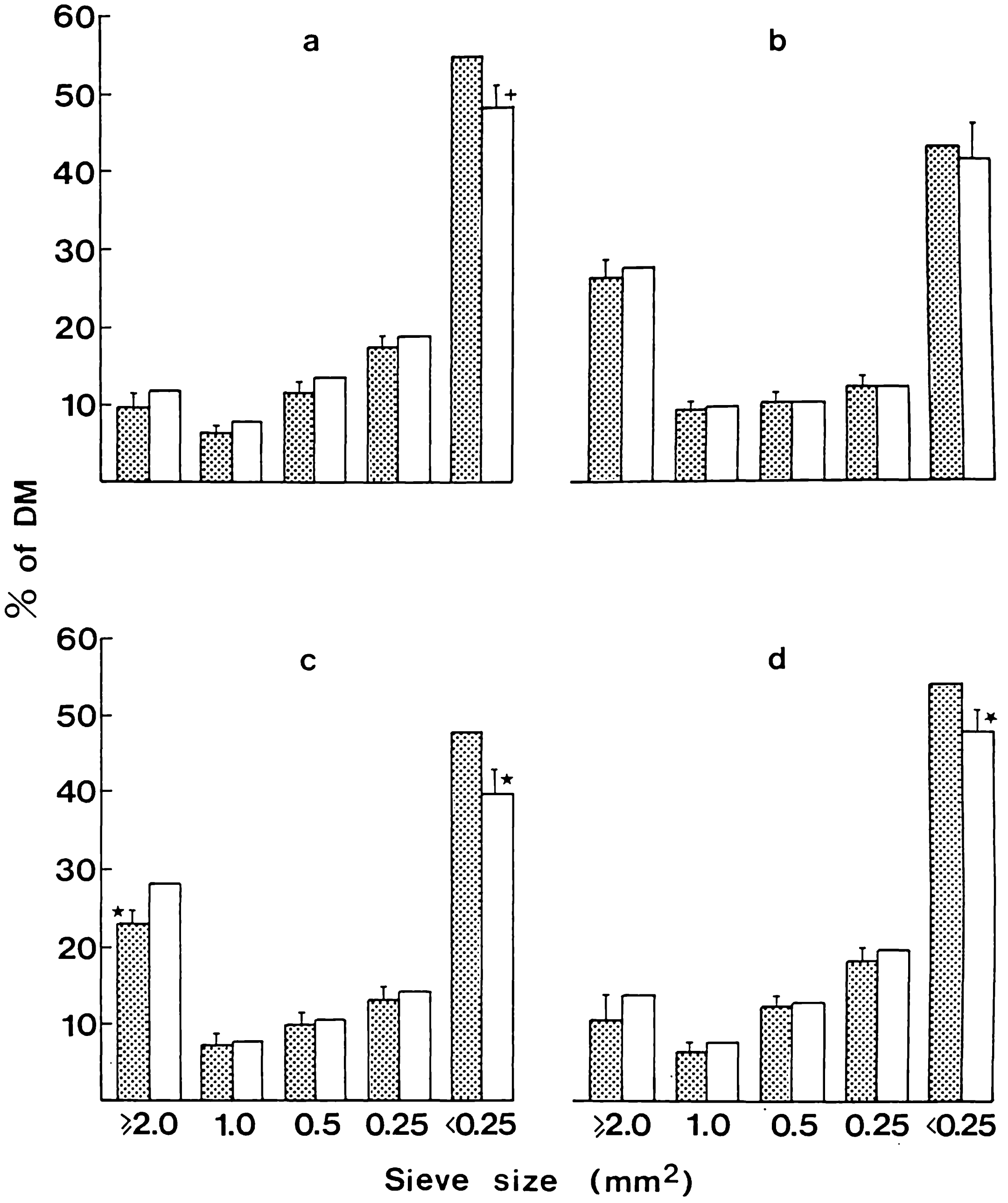


Figure 4.1 The percentage of DM in digesta retained on sieves for HS (▨) and LS (☐) cows before feeding (a) and at 0 (b), 7.5 (c) and 21 (d) h after feeding on pasture.

Table 4.5 The percentage of DM in the digesta of HS and LS cows[a] offered pasture (Experiment 5).

Sample time (h)		HS	LS	sed	sig.
Pre-feed		7.69	9.42	0.48	*
Post-feed	0[b]	11.16	11.51	0.25	ns
	7.5	9.89	11.22	0.44	*
	21	8.02	9.10	0.55	ns

[a] Data averaged for all treatments
[b] Sampled before treatments applied

Particle size of digesta in the reticulo-rumen

Particle size determinations were made for each cow when on the control treatment.

Seven fractions were identified, being sieve sizes 4, 2, 1, 0.5 and 0.25 mm, and residue and soluble material. In the data presented, material retained by the 4 and 2 mm sieves were combined because particles greater than 4 mm in length could pass through the 4 mm sieve lengthwise. Residue and soluble material were also combined and classed as material less than 0.25 mm length.

The percentage of DM in the sample retained on each sieve is shown in Figure 4.1. The 2 pre-feed sample results (Figure 4.1a,d) were similar, and indicated that digesta from HS cows had proportionately more material of less than 0.25 mm length than did digesta from LS cows. Feeding resulted in an increase in the proportion of large particles (>2 mm), from 10.7% to 26.8% of DM, and a decrease in particles less than 0.25 mm from 51.6% to 42.3% of DM (Figure 4.1b). There were no significant differences between susceptibility groups immediately after feeding. At 7.5 h (Figure 4.1c) HS cows had a lower proportion of particles 2 mm or greater (23.1% vs 28.5%) and more particles less than 0.25 mm (47.5% vs 39.5%)

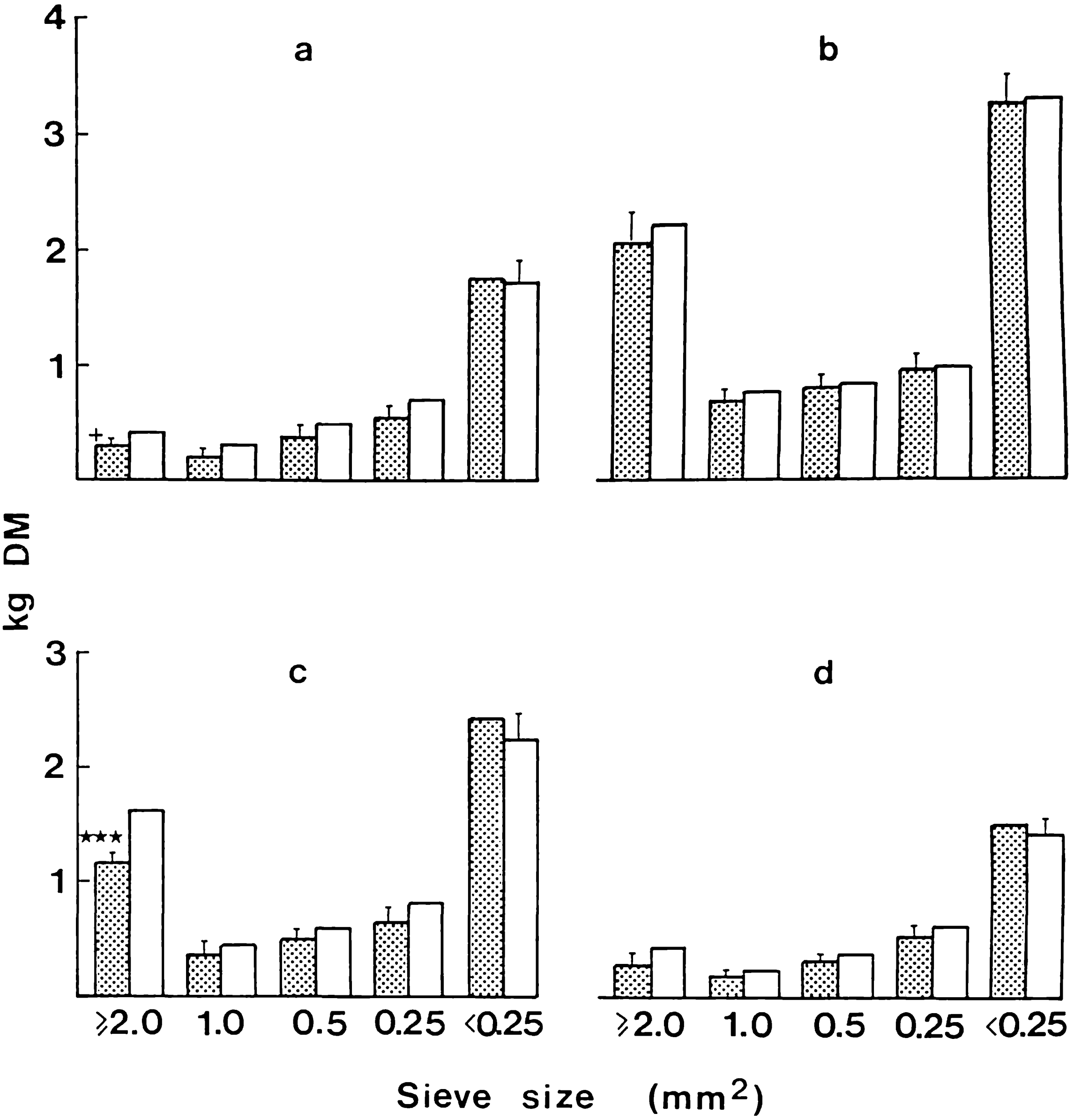


Figure 4.2 The quantity (kg) of DM in digesta retained on sieves for HS (▨) and LS (□) cows before feeding (a) and at 0 (b), 7.5 (c) and 21 (d) h after feeding on pasture.

than LS cows, the increase in particles less than 0.25 mm being due to the soluble proportion.

When expressed as kg DM in the reticulo-rumen (Figure 4.2) there were no significant differences between susceptibility groups in the quantity of each particle size before or immediately after feeding, except that HS cows had less DM greater than 2 mm than did LS cows before feeding (Figure 4.2a,b). Feeding increased the quantity of both large particles and fine material (Figure 4.2b). The quantity of particles greater than 2 mm was less in HS than LS digesta at 7.5 h (1.17 vs 1.62 kg, Figure 4.2c), but these differences were no longer apparent by the time of the next feeding (Figure 4.2d). The quantity of DM in the reticulo-rumen was lower at 21 h (Figure 4.2d) than before feeding (Figure 4.2a) because the data in Figure 4.2d were not adjusted for losses by sampling.

Reticulo-rumen capacity

LS cows had 5.5 kg greater reticulo-rumen capacity than did HS cows (Table 4.6). Capacity, as a percent of live weight, averaged 25% for both susceptibility groups.

Digesta fill was 6 percentage units lower for LS than HS cows before feeding, but was similar for both susceptibility groups after feeding (Table 4.6).

Table 4.6 Water-filled reticulo-rumen capacity and digesta quantity expressed as a proportion of capacity for HS and LS cows offered pasture (Experiment 5).

		HS	LS	sed	sig.
Capacity (kg)		95.7	101.2	5.4	ns
Fill (%)	pre-feed	43.0	37.0	2.5	*
	post-feed	71.8	69.3	2.4	ns

4.3.2 Experiment 6 - Restricted feeding of pastures of low, medium and high maturity

Diet composition

With increasing pasture maturity the contents of DM and fibre increased and the digestibility *in vitro* and concentrations of nitrogen, K and P decreased (Table 4.7).

Table 4.7 DM content (%), energy content (MJ/kg DM), digestibility *in vitro* (%) and concentrations (g/100 g DM) of MAD fibre, water-soluble sugar, nitrogen (N) and minerals in pasture diets (Experiment 6).

	Pasture maturity		
	L[a]	M	H
DM	13.7	15.4	20.3
Energy	18.81	18.12	18.14
Digestibility	73.37	69.21	67.63
MAD fibre	25.32	29.88	32.80
Sugar	3.10	2.39	2.83
N	3.45	2.07	1.68
Ca	0.73	1.24	0.61
Mg	0.21	0.11	0.17
Na	0.08	0.09	0.06
K	3.78	3.42	2.68
P	0.39	0.32	0.27

[a] In this and subsequent tables:
L - low, M - medium, H - high maturity

Live weight

LS cows were 55 kg heavier than HS cows (462 and 407 kg respectively, sed 8, P<0.001). The cows were, on average, 32 kg heavier than when used in Experiment 5.

Intake and the quantity of digesta in the reticulo-rumen

On measurement days the intakes of fluid and DM were slightly higher for LS than HS cows (Table 4.8) due to the influence of live weight on DM allowance. DM intake was higher on low maturity pasture

Table 4.8 Intakes (kg/cow) on measurement days, and the quantities (kg) removed from the reticulo-rumen before and after feeding and calculated net losses (kg) of fluid, DM and total digesta for HS and LS cows offered 3 pasture diets (Experiment 6).

	Pasture maturity[a]					Susceptibility[b]			
	L	M	H	sed	sig.	HS	LS	sed	sig.
Fluid									
Intake	29.87	22.61	16.26	0.25	***	21.94	23.89	0.56	*
Digesta									
pre-feed	33.01	39.16	43.50	0.90	***	39.79	37.32	1.66	ns
post-feed	59.09	59.13	59.33	1.11	ns	57.75	60.61	1.69	ns
loss[c] 1	1.41	-0.68	-1.89	0.58	***	1.00	-1.77	1.13	+
2	23.50	18.41	15.31	0.94	***	17.08	21.06	1.12	*
DM									
Intake	5.17	4.99	5.00	0.06	*	4.83	5.28	0.13	*
Digesta									
pre-feed	1.80	2.18	2.37	0.10	***	2.03	2.20	0.16	ns
post-feed	6.45	6.70	7.08	0.20	*	6.34	7.14	0.19	**
loss 1	0.38	0.31	0.16	0.17	ns	0.36	0.20	0.17	ns
2	4.67	4.01	4.38	0.23	*	4.09	4.61	0.13	**
Total									
Intake	35.04	27.60	21.26	0.31	***	26.77	29.17	0.69	*
Digesta									
pre-feed	34.81	41.34	45.87	0.97	***	41.82	39.52	1.69	ns
post-feed	65.54	65.83	66.41	1.21	ns	64.09	67.75	1.85	+
loss 1	1.79	-0.37	-1.73	0.53	***	1.36	-1.57	0.99	*
2	28.17	22.42	19.69	1.02	***	21.17	25.67	1.13	**

[a] Averaged for all cows
[b] Averaged for all pasture maturities
[c] 1 - during feeding; 2 - during 0-21.5 h after feeding

than on medium or high maturity pasture. Intake of fluid in the food decreased as maturity increased because the DM content of the feed increased with increasing maturity. Even when drinking water was available on non-measurement days there was a significant, though less marked, difference between pasture maturities in their effect on water intake (33.13, 29.72, 28.01 kg/cow/day, sed 1.13, $P<0.001$, for L, M and H respectively). Averaged for all pasture maturities, HS and LS cows consumed, respectively, 29.14 and 31.43 kg water/cow/day (sed 2.40, ns), of which 6.4 kg was consumed by drinking for both susceptibility groups. DM intakes were similar on non-measurement and measurement days.

There were significant effects of pasture maturity on the quantities and losses of digesta, particularly fluid (Table 4.8). As pasture maturity increased there was an increase in the quantity of fluid (and total digesta) in the reticulo-rumen before feeding, but no change in post-feeding quantity. Losses over both the feeding and post-feeding periods decreased as maturity increased, reflecting the changes in intake. There were no interactions between pasture maturity and susceptibility group for quantities of fluid and total digesta; averaged for all maturities LS cows showed a net gain over the feeding period compared to a loss for HS cows, and LS cows lost more over the subsequent 21.5 h than did HS cows.

The quantity of DM in the reticulo-rumen both before and after feeding increased with increasing pasture maturity. There was no effect of maturity on loss of DM over the feeding period and an inconsistent effect on 21.5 h loss, being lower on M than on L or H. There were significant interactions between pasture maturity and susceptibility group in the quantities of DM removed before and after feeding and in 21.5 h loss, but no consistent trend accounted for these interactions. When averaged across all maturities LS cows had significantly more DM in the reticulo-rumen after feeding, and lost more following feeding, than did HS cows.

There were significant, but inconsistent, effects of pasture maturity on digesta DM content at the sample times after feeding (Table 4.9), although immediately after feeding the DM content was highest on the most mature pasture when the water intake was lowest. LS cows maintained a significantly higher digesta DM content than HS cows after feeding. The DM contents obtained 21.5 h after feeding were similar to those obtained before feeding, despite different water intakes in their respective previous 24 h. The difference between susceptibility groups in DM content was significant at 21.5 h, but not before feeding, due to a smaller sed rather than to a larger difference between susceptibility groups.

Table 4.9 The percentage of DM in the digesta of HS and LS cows offered 3 pasture diets (Experiment 6)

Sample time (h)	Pasture maturity[a] L	M	H	sed	sig.	Susceptibility[b] HS	LS	sed	sig.
Pre-feed	5.18	5.33	5.16	0.19	ns	4.85	5.59	0.40	ns
Post-feed 0	9.83	10.18	10.64	0.24	*	9.89	10.54	0.16	**
21.5	4.46	6.03	5.58	0.26	***	4.95	5.76	0.14	**

[a] Averaged for all cows
[b] Averaged for all pasture maturities

4.4 DISCUSSION

The results of Experiment 5 showed that increasing or decreasing the quantity of digesta in the reticulo-rumen correspondingly increased or decreased the quantity of digesta lost from the reticulo-rumen over the next 21 h. There were also corresponding changes in the quantity of digesta remaining at 21 h, but the changes in quantity of digesta at 21 h were smaller than those in the quantity lost from the reticulo-rumen.

Altering the quantity of digesta in the reticulo-rumen by addition or subtraction via the rumen fistula after feeding may have resulted in effects on saliva production, reticulo-ruminal motility and digesta composition, which differed from those occurring when the quantity altered through the animal's own intake. However, Campling *et al.* (1961) and Ulyatt *et al.* (1984) have observed increases in the quantity of digesta before and after feeding in response to an increased level of intake. Of interest here is the finding that the regression coefficients did not differ between cows of either susceptibility group, indicating that the quantity of digesta lost from the reticulo-rumen per unit increase in the quantity of digesta in the reticulo-rumen was similar for both groups. In contrast to the data obtained on pasture in Experiment 3, however, there were no differences between susceptibility groups in intercept values for the regression equations and, therefore, in the quantity of digesta in the reticulo-rumen at 21 h, although digesta fill was lower in LS than HS cows because of their larger reticulo-rumen capacity.

Differences between Experiments 3 and 5 in the pasture diets may have contributed to the different results observed. The pasture consumed in Experiment 5 was intermediate between the hay and pasture diets in Experiment 3 in digestibility *in vitro*, but was similar to the pasture in other constituents measured. Experiment 6 investigated the effect of maturity or digestibility of the diet on the quantity of digesta in the reticulo-rumen. Bloat is often associated with immature, rapidly growing pasture (Hancock 1953; Clarke & Reid 1974) and the most immature pasture was expected to be similar to that which might cause bloat. Digesta was foamy when cows were offered low maturity pasture but not when offered pasture of medium or high maturity, although no bloat occurred on any of the pastures.

Data in Experiment 6 showed that as pasture maturity increased (and digestibility decreased), the quantity of both fluid and DM in the reticulo-rumen before feeding increased. Presumably the quantity of fluid in the reticulo-rumen after feeding would also have increased, as occurred for DM, had water not been withheld. Diets which are low in digestibility are retained longer in the reticulo-rumen (Blaxter *et al*. 1961; Campling *et al*. 1961), but many studies which have included measurements of the quantity of digesta when comparing diets differing in digestibility have confounded differences in digestibility and intake, since voluntary intakes are reduced on diets of low digestibility (Blaxter *et al*. 1961; Campling *et al*. 1961, Ulyatt *et al*. 1967). However, results of Ulyatt (1969) and Egan *et al*. (1975) also suggested that, at similar DM intakes, the quantity of digesta in the reticulo-rumen increased with decreasing digestibility of the diet. In the present experiment both susceptibility groups increased their quantity of digesta with increasing pasture maturity, and the quantity of digesta in the reticulo-rumen was similar for HS and LS cows even on diets of higher digestibility *in vitro* than that of the pasture in Experiment 3.

Water intakes were similar for HS and LS cows on all pastures on non-measurement days in Experiment 6, unlike those measured on pasture diets in Experiments 3 and 5. In all experiments, however, LS cows maintained slightly higher DM contents of digesta than did HS cows. In both Experiments 5 and 6, DM contents of digesta before feeding were similar whether water had or had not been available in the previous 24 h, and the relative DM contents of HS and LS cows' digesta were not affected by withholding water. This suggests that water loss from the reticulo-rumen after feeding changed according to intake, independently of DM loss. There was no difference between the quantities of digesta removed before feeding when drinking water had been available and those when water had been withheld in the previous feeding period (Table 4.4), suggesting that the decision to withhold water did not influence subsequent relative quantities of digesta in the HS and LS cows.

The percentages of DM in digesta in Experiment 6 were considerably lower than those found in Experiments 3 and 5, yet DM content of digesta before feeding was not affected by pasture maturity. The lower DM content in Experiment 6 appeared to result from less DM in the reticulo-rumen relative to that in Experiments 3 and 5, rather than more fluid and therefore an increase in total quantity of digesta. This implies a lower retention time of DM and, therefore, more rapid degradation and loss from the reticulo-rumen in Experiment 6. Thus, despite similar values for digestibility in vitro, the pasture diets may have differed in digestibility and retention time in the reticulo-rumen.

The difference between susceptibility groups in water intake observed in Experiments 3 and 5 was possibly associated with the higher DM content of digesta, compared to Experiment 6.

The data obtained in Experiment 6 indicated that the net loss of digesta from the reticulo-rumen during feeding decreased as the pasture maturity increased. This may have been due to increased salivary input, as Meyer et al. (1964) reported that saliva production during feeding increased as the DM content of the feed increased, although Bailey (1961a) and Carr (1984) failed to find this effect.

Data obtained in both Experiments 5 and 6 indicated that net losses of fluid from the reticulo-rumen during feeding were greater, and after feeding were lower, for HS compared to LS cows. The mechanisms by which this could arise are discussed in Chapter 7.

The changes in particle size with time after feeding obtained in Experiment 5 were similar to previously reported results (Pearce 1967; Evans et al. 1973; Reid et al. 1979). The present study provides only preliminary data on the particle sizes in digesta of HS and LS cows. Distribution of both DM and size of particles varies in sites around the rumen and reticulum (Evans et al. 1973), therefore bulk sampling of digesta for particle size analysis could be misleading.

It is generally accepted that a critical size of particle is required for escape from the reticulo-rumen (Ulyatt et al. 1976; Reid et al. 1977; Poppi et al. 1980), although size alone does not determine rate of escape. The data obtained suggested that HS cows had a greater proportion of fine (<0.25 mm) material before feeding. Clarke & Reid (1974) also reported that digesta from HS cows had a higher concentration of finely divided particulate matter than did that from LS cows. Majak et al. (1983) found an increased buoyancy of particulate matter in pre-feed digesta from cows which subsequently bloated, although this may not necessarily reflect a greater content of particulate matter. The present differences between susceptibility groups were small, suggesting that differences in particle size were unlikely to be contributing to or associated with differences in the pattern of loss of digesta between HS and LS cows. Since there was no difference between susceptibility groups in the quantity of digesta at 21 h in Experiment 5, any association between particle size characteristics and the quantity of digesta could not be assessed. Further investigations of particle size were not considered to be warranted until the relationships between susceptibility, diet and quantity of digesta were clarified.

There were several other differences between Experiments 3 and 5, in addition to dietary factors, which may have influenced the results. These included the Na status of the cows, the presence or absence of pregnancy, and the level of DM intake. The latter two factors are discussed in Chapter 7 when results from all experiments are considered. Na status in Experiment 5 (and 6) was assumed to be adequate as cows had access to NaCl block prior to the experiment and were supplemented with NaCl during the experiment. In an additional experiment, an attempt was made to measure the quantities of digesta in the reticulo-rumen before and after feeding when the cows were Na-deplete and Na-replete. Depletion was attempted by removal of 10-15 kg rumen fluid at 2-3 day intervals, combined with restricted grazing that allowed intakes of about 4 kg DM per cow per day. The concentrations of Na and K in rumen fluid and saliva were monitored. A decrease in the concentration of Na in rumen fluid from 130 mmol/ℓ to 80 mmol/ℓ, and a shift in the Na:K ratio in saliva from 15-20 to less than 6, was achieved over 21 days. However, the concentration of Na in rumen fluid and the Na:K ratio in saliva were elevated to pre-depletion levels within 5 days of feeding at a maintenance level when making digesta measurements. This showed that recovery from a Na depleted state can be extremely rapid, as has been previously reported (Denton 1956). It also suggested that the depleted state observed in

Experiment 3 was the result of a long term low Na intake, combined with some leakage from the fistula.

The rapid Na repletion led to the experiment being abandoned and the effects of Na status on the quantity of digesta in the reticulo-rumen were not investigated. An alternative approach was adopted to experimentally examine the effect of the concentration of Na in reticulo-rumen digesta on digesta parameters. The next chapter describes this work.

CHAPTER 5

THE EFFECT OF INTRARUMINAL LOADING WITH WATER AND ELECTROLYTES ON THE QUANTITY AND COMPOSITION OF DIGESTA OF COWS DIFFERING IN SUSCEPTIBILITY TO BLOAT

5.1 INTRODUCTION

Data obtained in Experiment 3 suggested that the greater post-feeding loss of digesta and lower quantity of digesta in the reticulo-rumen 21 h after feeding in LS cows than HS cows when offered pasture, but not when offered hay, may have been associated with a lower concentration of Na in the rumen fluid of LS cows. Experiment 7 described below investigated the effect of the Na concentration of rumen fluid on losses of fluid and DM from the reticulo-rumen.

5.2 MATERIALS AND METHODS

5.2.1 Cows

The 8 cows were those used in Experiments 5 and 6. They were neither lactating nor pregnant and were housed as described in Chapter 3.2.1.

The cows were prevented from cycling in oestrus during the experimental period by the use of intravaginal CIDR (controlled internal drug release) dispensers consisting of a silicone elastomer impregnated with 1.7 g (18% w/w) progesterone (Alex Harvey Industries, Hamilton, New Zealand).

5.2.2 Feeding, experimental design and measurements

Feeding Chaffed perennial ryegrass-white clover hay was offered at a maintenance feeding level (see Chapter 3.2.2) for 3 h each day. Chemical composition of the diet is given in Table 5.1. Water was offered to appetite after 1.5 and 3 h of feeding. The cows were managed in 2 groups (2 HS, 2 LS per group) which differed in feeding and measurements by 45 minutes. Food was offered at 1430 and 1515 h.

Table 5.1 Energy content (MJ/kg DM), digestibility *in vitro* (%) and concentrations (g/100 g DM) of MAD fibre, water-soluble sugar, nitrogen (N) and minerals in hay.

Variate	Value
Energy	18.41
Digestibility	64.30
MAD fibre	34.73
Sugar	9.27
N	1.50
Ca	0.47
Mg	0.13
Na	0.22
K	1.73
P	0.26

Experimental design The experiment consisted of 13 days adaptation followed by a 14-day experimental period, when 4 treatments were imposed on 4 HS-LS pairs of cows in a Latin square arrangement. At least 3 days were allowed between successive treatments. The treatments commenced 15 h after the end of the feeding period, by which time the osmolality and electrolyte concentrations of digesta in the reticulo-rumen were expected to be stable.

The treatment solutions are shown in Table 5.2. Solution L consisted of 10 ℓ distilled water. Solution C contained Na and K in concentrations similar to those in parotid saliva (McDougall 1948). Solutions H and HH were twice and 4 times the concentration of solution C, respectively. Solutions L, C, H and HH contained 0, 1.76, 3.52 and 7.04 moles Na, respectively. The pH of the electrolyte solutions was 8.2.

Each 10 ℓ solution contained 2.65 g Cr, as Cr EDTA.

Table 5.2 Composition (g/10 ℓ water) of solutions added to digesta.

Solution	$NaHCO_3$	$Na_2HPO_4.2H_2O$	NaCl	KCl
L	0	0	0	0
C	98.3	46.2	4.7	5.7
H	196.5	92.4	9.4	11.4
HH	393.0	184.8	18.8	22.8

Measurements On each measurement day digesta in the reticulo-rumen was removed from each cow, weighed, sampled for DM content and replaced 15 h after the feeding period (0 h) and at 1.5 and 7.5 h later. The treatment solutions were warmed to 39 °C and were mixed with digesta at 0 h, prior to its return to the reticulo-rumen. Rumen fluid was sampled from digesta at 1, 0.5 and 0 h before addition of solutions and at 0, 0.75, 1.5 h and thereafter at hourly intervals until 7.5 h after addition of solutions. Fluid samples were analysed for osmolality and concentrations of Na and K, and those taken after addition of solutions also analysed for Cr concentration.

The pH of digesta in the ventral rumen was measured at each sampling time.

Addition to experimental period Four days after completion of the Na and water loading treatments, the measurement procedure was repeated (with the exception of dilution of Cr) on 2 HS and 2 LS cows with the addition to digesta of 5 ℓ distilled water containing 179 g KCl (0.48 M). This amount was calculated to increase the concentration of K in rumen fluid to 50-60 mmol/ℓ, similar to that observed in Experiment 3 (restricted feeding of hay and pasture to non-lactating cows).

5.2.3 Techniques and sampling procedures

Removal and sampling of digesta for DM and fluid analyses have been previously described (Chapter 3.2.3). The pH of a 250 ml sample of digesta withdrawn from the ventral rumen was measured using a pH meter (Philips PW 9409) situated near the cows.

5.2.4 Sample analysis

The composition of a freeze-dried sample of the hay offered was determined as described in Chapters 2.2.5 and 3.2.4. The DM content of digesta and the osmolality and concentrations of Na, K and Cr in rumen fluid were determined as described in Chapter 3.2.4.

5.2.5 Calculations and statistical analysis

Digesta losses over 90 and 360 minutes were calculated as described in Chapter 3.2.5. The total loss over 0-7.5 h was calculated as the sum of losses over 0-1.5 h and 1.5-7.5 h without adjustment for samples removed at 1.5 hours.

The rumen fluid dilution rates and the net inflows to and outflows from the reticulo-rumen were calculated as described in Chapter 3.2.5.

Analysis of variance models provided by Genstat V were used to test for differences between susceptibility groups and treatments, and covariance analysis was used to describe relationships between variables.

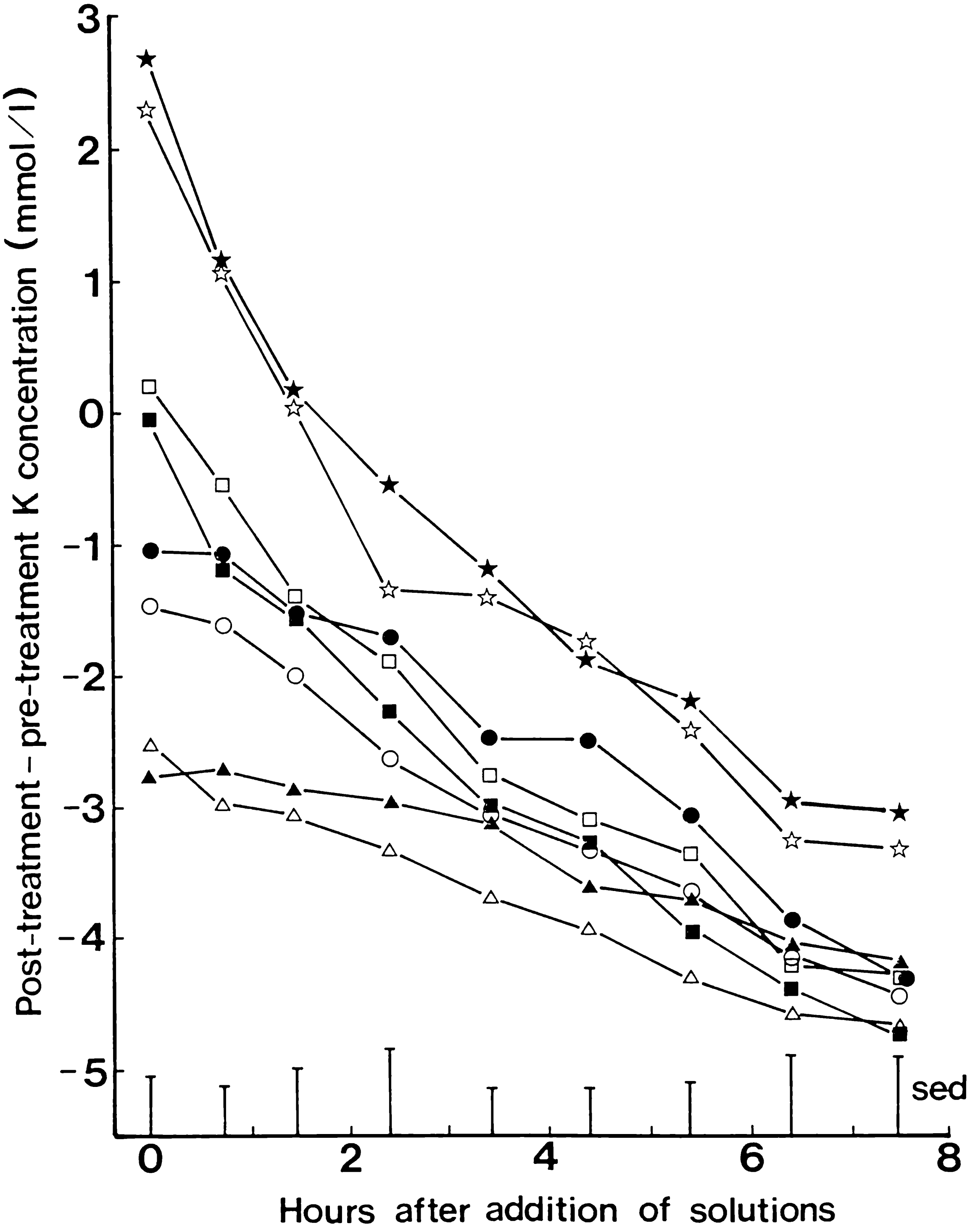


Figure 5.3 The effect of intraruminal addition (post-treatment minus pre-treatment) of water (▲,△) and electrolyte solutions C (●,○), H (■,□) and HH (★,☆) on the K concentration of rumen fluid from HS cows (closed symbols) and LS cows (open symbols).

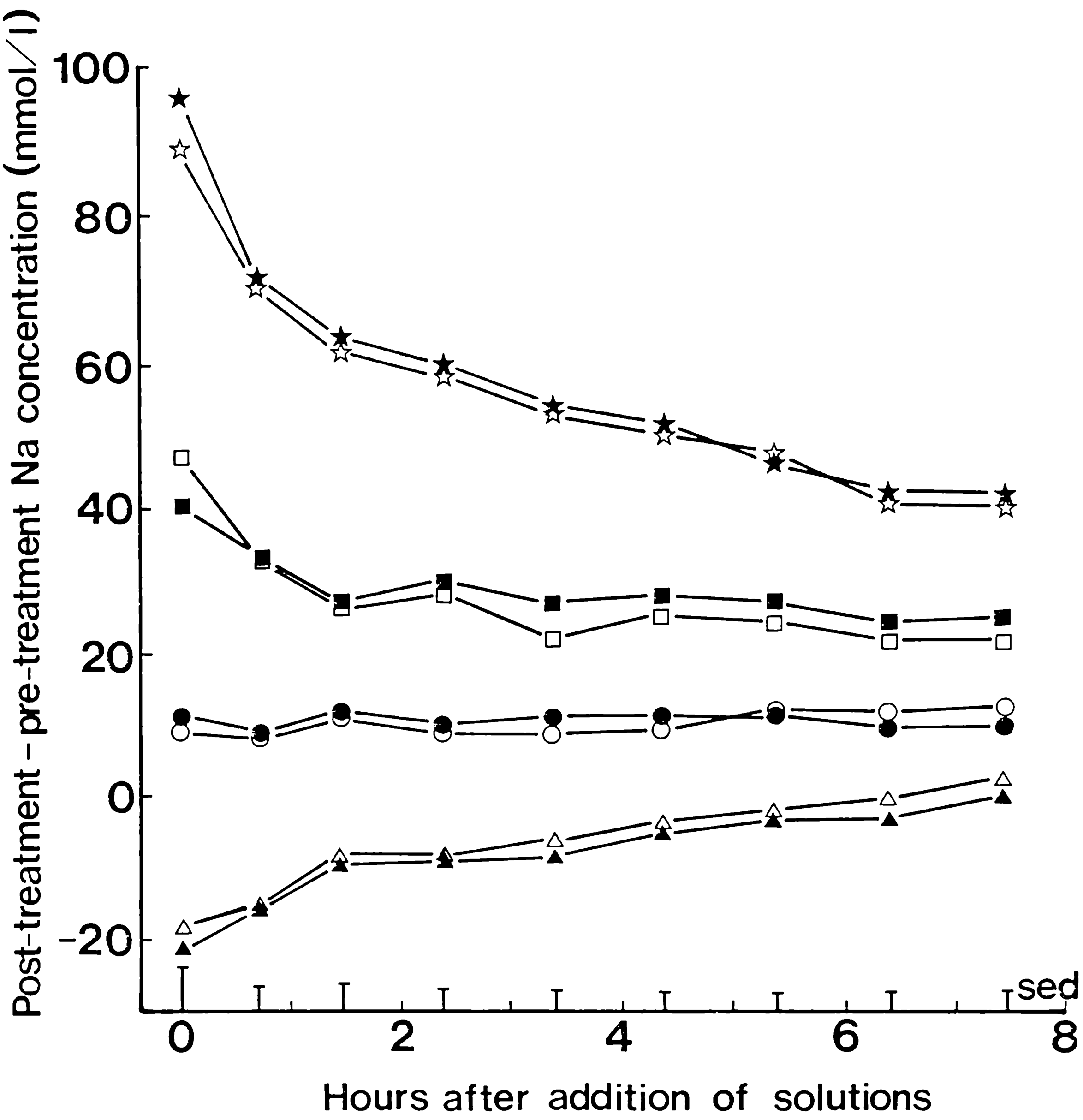


Figure 5.2 The effect of intraruminal addition (post-treatment minus pre-treatment) of water (▲,△) and electrolyte solutions C (●,○), H (■,□) and HH (★,☆) on the Na concentration of rumen fluid from HS cows (closed symbols) and LS cows (open symbols).

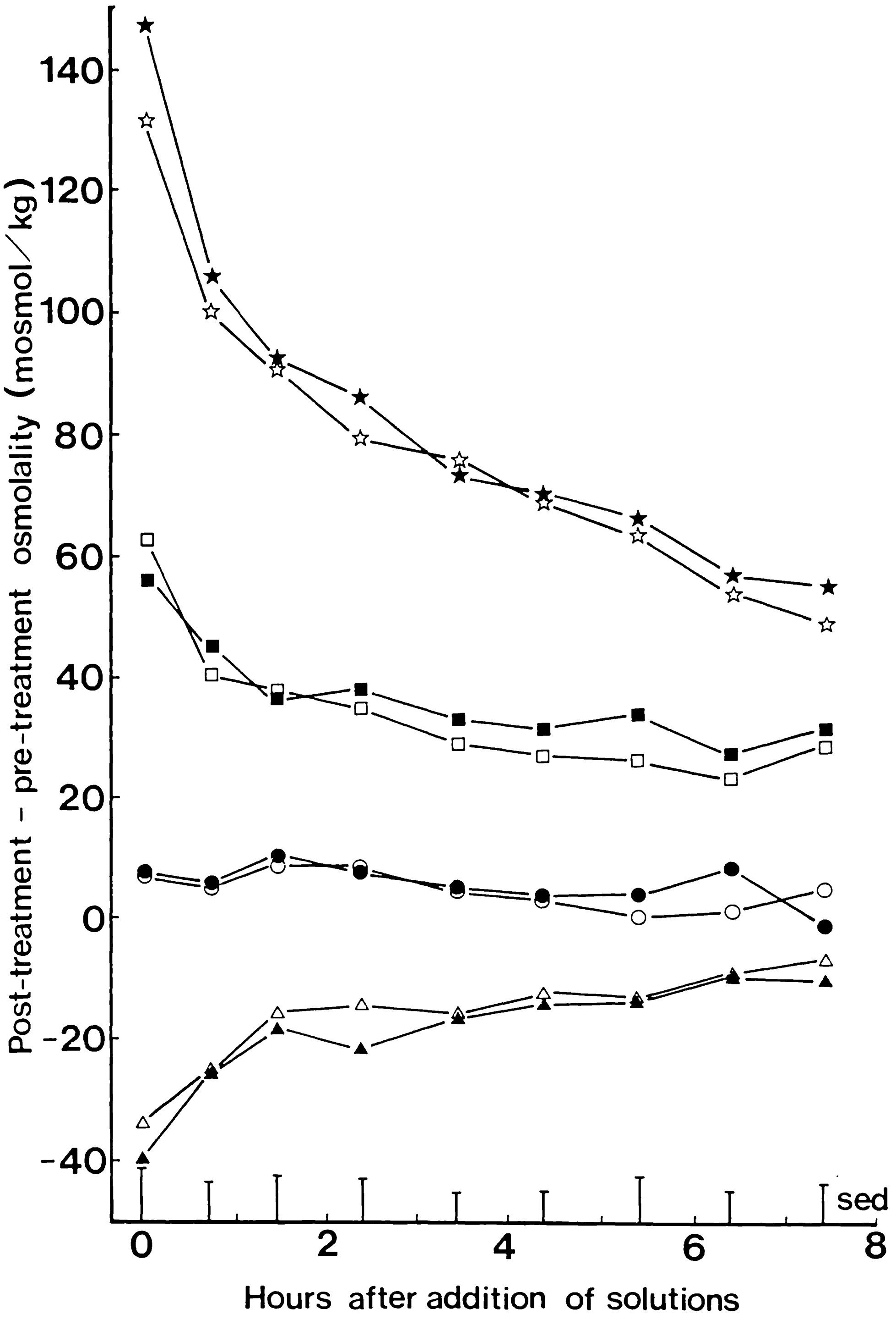


Figure 5.1 The effect of intraruminal addition (post-treatment minus pre-treatment) of water (▲,△) and electrolyte solutions C (●,○), H (■,□) and HH (★,☆) on the osmolality of rumen fluid from HS cows (closed symbols) and LS cows (open symbols).

5.3 RESULTS

5.3.1 Osmolality, Na and K concentrations

Osmolality and Na and K concentrations of rumen fluid prior to the addition of treatment solutions did not differ between susceptibility groups and averaged (± standard error) 275 mosmol/kg (±2), 130 mmol/ℓ (±1) and 14.3 mmol/ℓ (±0.4), respectively. The effects of treatment on concentrations are shown in Table 5.3.

Table 5.3 Rumen fluid osmolality (mosmol/kg) and concentrations of Na and K (mmol/ℓ) following intraruminal addition of water (L) and electrolyte solutions (C, H, HH) to HS and LS cows.

	L		C		H		HH			
	HS	LS	HS	LS	HS	LS	HS	LS	sed[a]	sig.
Osmol	236	243	285	283	325	337	425	407	11	ns
Na	109	111	141	140	167	178	230	220	6	ns
K	10.8	11.7	12.4	12.7	16.1	14.6	16.5	16.7	1.0	ns

[a] Standard error of the difference for the interaction between susceptibility group and treatment

There were no differences between susceptibility groups in the rates of return of osmolality or concentrations of Na and K towards pre-treatment values (Figures 5.1, 5.2, 5.3).

Osmolality and Na concentration were elevated by addition of solution C and the Na concentration remained elevated over the sampling period. Addition of water alone (L) decreased osmolality and Na concentration but these subsequently increased over 7.5 h to values which were similar to (Na) or just below (osmolality) those obtained prior to treatment addition. Osmolality and Na concentration were increased by addition of solutions H and HH, and although values subsequently decreased they remained elevated above pre-treatment values at 7.5 h. Concentrations of K were decreased by the addition of solutions L and C and increased by the addition of solutions H and

Table 5.4 Losses (kg) of fluid, DM and total digesta after intraruminal addition of water (L) and electrolyte solutions (C, H, HH) to HS and LS cows.

	Treatment[a]						Susceptibility[b]			
	L	C	H	HH	sed	sig.	HS	LS	sed	sig.
Fluid										
0-1.5 h	1.65	1.77	0.72	-0.52	0.52	***	0.61	1.19	0.66	ns
1.5-7.5 h	6.81	6.29	5.53	3.53	0.73	***	4.33	6.75	0.91	*
0-7.5 h	8.46	8.06	6.25	3.01	0.76	***	4.94	7.94	1.08	*
DM										
0-1.5 h	1.05	0.65	0.51	0.39	0.37	ns	0.46	0.85	0.20	+
1.5-7.5 h	0.80	0.71	0.80	1.03	0.26	ns	0.87	0.80	0.21	ns
0-7.5 h	1.85	1.36	1.31	1.42	0.27	ns	1.33	1.65	0.16	ns
Total										
0-1.5	2.70	2.42	1.23	-0.13	0.45	***	1.07	2.04	0.57	ns
1.5-7.5 h	7.61	7.00	6.33	4.56	0.75	**	5.20	7.55	0.81	*
0-7.5 h	10.31	9.42	7.66	4.43	0.68	***	6.27	9.59	1.06	*

[a] Averaged for all cows

[b] Averaged for all treatments

HH, but concentrations subsequently decreased on all treatments. The average reductions in K concentration over the 7.5 h period were 1.8, 3.1, 4.5 and 5.7 mmol/ℓ for solutions L, C, H and HH, respectively.

5.3.2 Digesta loss

Treatment had a significant effect on the quantity of digesta lost from the reticulo-rumen over 7.5 h (Table 5.4). The net loss of fluid, but not DM, decreased with increasing Na concentration, and the effect was apparent after both 1.5 and 7.5 h. When averaged across all treatments, the LS cows lost significantly more fluid, but not DM, than HS cows over the 7.5 h (Table 5.4). This difference in fluid loss was consistent for all treatments. The relationship between the change over 7.5 h in quantity of fluid and the change over 7.5 h in concentration of Na was tested for differences in slope and intercepts between susceptibility groups. There was no difference between HS and LS cows in the slope of the regression; at the average slope LS cows lost more fluid than did HS cows for any given shift in Na concentration (Table 5.5).

The quantity of fluid returned to the reticulo-rumen after addition of solutions to digesta was slightly greater for LS than for HS cows (66.7 and 63.8 kg, respectively, sed 2.5, ns), but there was no relationship between the quantity of fluid returned and the subsequent fluid loss over 7.5 h, as indicated by the regression coefficient (Table 5.5).

Table 5.6 The quantities (moles) of Na and K in the reticulo-rumen after intraruminal addition of water (L) or electrolyte solutions (C, H, HH) to HS and LS cows, and subsequent losses (moles) over 1.5 and 7.5 h.

	Treatment[a]						Susceptibility[b]			
	L	C	H	HH	sed	sig.	HS	LS	sed	sig.
Na										
Quantity	7.09	9.07	11.41	14.75	0.33	***	10.35	10.80	0.34	ns
Loss 0-1.5	-0.44	0.27	1.16	1.85	0.32	***	0.65	0.77	0.20	ns
1.5-7.5	0.16	0.79	1.28	2.10	0.10	***	0.91	1.25	0.19	ns
K										
Quantity	0.73	0.81	1.01	1.10	0.03	***	0.89	0.93	0.06	ns
Loss 0-1.5	0.04	0.06	0.12	0.15	0.03	***	0.08	0.10	0.02	ns
1.5-7.5	0.15	0.21	0.26	0.25	0.02	***	0.20	0.23	0.01	***

[a] Averaged for all cows
[b] Averaged for all treatments

Table 5.5 Pooled regression coefficients (b ± standard error) and intercepts for relationships between the change over 7.5 h in fluid quantity (kg) and either the change in Na concentration over 7.5 h (Δ Na, mmol/ℓ) or the quantity of fluid returned after addition of solutions to digesta (Ret 0, kg).

x-variate	b	± se	sig.	HS	LS	sed	sig.
Δ Na	-0.070	0.011	***	-5.87	-8.83	1.15	**
Ret 0	0.050	0.285	ns	-7.77	-11.64	0.96	**

5.3.3 Changes in amounts of Na and K in digesta

The amount of K but not of Na in digesta differed significantly between treatment groups before treatment addition, being higher prior to addition of solution H than of water alone ($P<0.05$). The amounts of both Na and K differed between treatments after addition of solutions (Table 5.6). There was a net gain in the amount of Na in digesta over 1.5 h after addition of water (L), compared to losses after addition of solutions C, H and HH. There were net losses of K on all treatments.

There were no significant differences between susceptibility groups or interactions between susceptibility group and treatment, except that LS cows lost slightly but significantly more K over 1.5-7.5 h than did HS cows ($P<0.001$) when averaged across all treatments.

5.3.4 Net fluid inflow to and outflow from the reticulo-rumen

Treatment had a significant effect on both net fluid inflow to the reticulo-rumen and outflow to the omasum (Table 5.7). Net inflow was lower after addition of solution HH than of C, and outflow was lower after addition of solution HH than of water alone or solutions C or H. Thus the lower net fluid loss with solution HH was due to a lower outflow rather than to an increased net inflow, and Na concentration decreased by absorption rather than by dilution.

There were no significant differences between the susceptibility groups in net inflow to or outflow from the reticulo-rumen, although LS cows tended to have slightly higher net inflow and outflow rates than did HS cows. This suggested that the greater net loss of fluid by LS cows resulted from an increase in outflow rather than a decrease in net inflow.

Table 5.7 Calculated net inflow to the reticulo-rumen and outflow to the omasum (ℓ/h) after intraruminal addition of water (L) or electrolyte solutions (C, H, HH) to HS and LS cows.

	Treatment[a]						Susceptibility[b]			
	L	C	H	HH	sed	sig.	HS	LS	sed	sig.
Net inflow	4.50	5.09	4.76	4.31	0.29	+	4.47	4.86	0.38	ns
Outflow	5.63	6.17	5.60	4.71	0.29	**	5.13	5.92	0.47	ns

[a] Averaged for all cows
[b] Averaged for all treatments

5.3.5 DM content and pH of digesta

The DM content of digesta was significantly lower at 7.5 h after addition of solution HH than of water alone (Table 5.8). There were no differences between susceptibility groups in digesta DM content before or after addition of solutions.

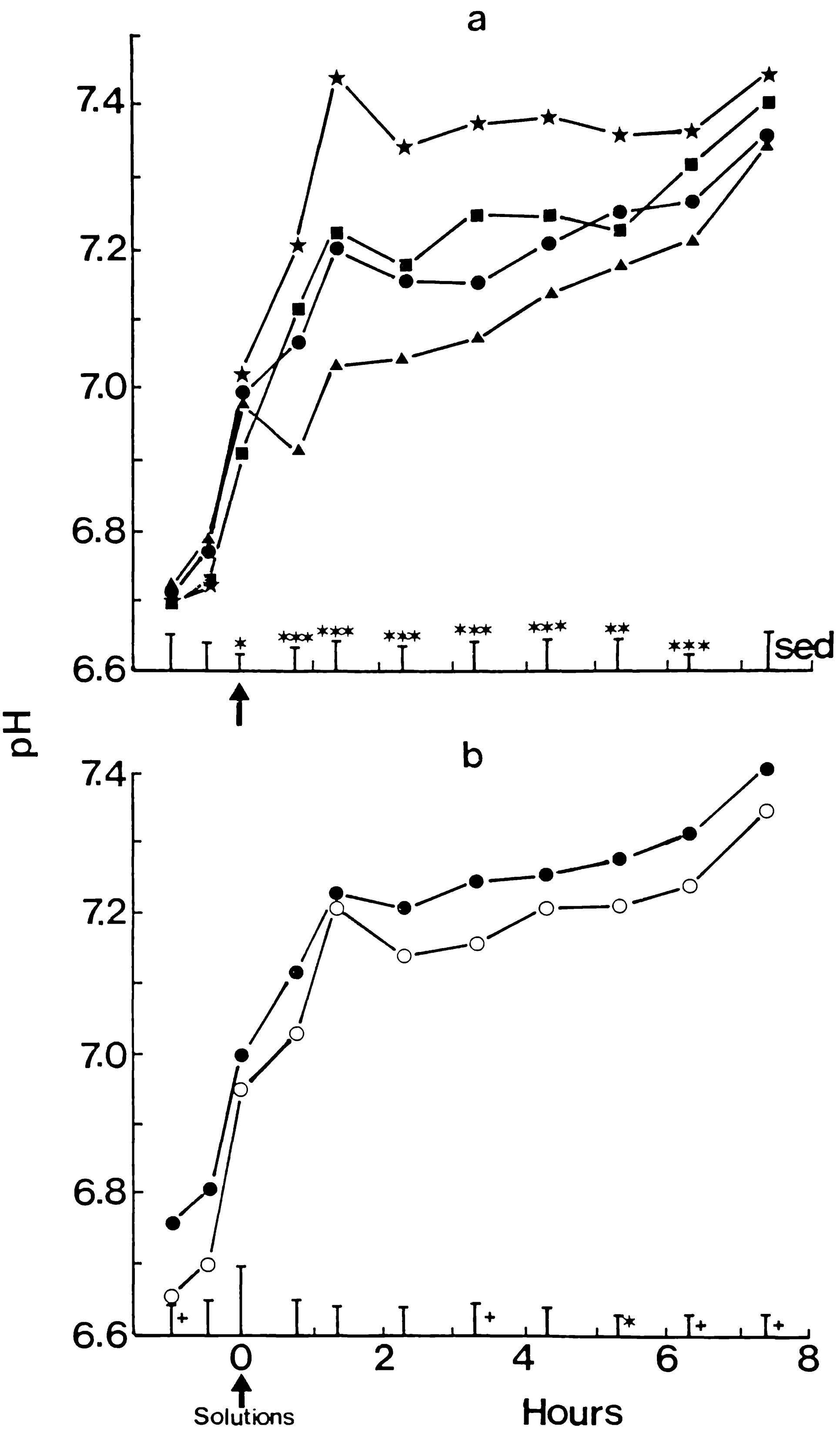


Figure 5.4 The pH of rumen fluid (a) averaged for both susceptibility groups after intraruminal addition of water (▲) and electrolyte solutions C (●), H (■) and HH (★) and (b) averaged for treatment solutions for HS (●) and LS (○) cows.

Table 5.8 The percentage of DM in the digesta of HS and LS cows before and after intraruminal addition of water (L) or electrolyte solutions (C, H, HH).

Sample time	(h)	Treatment[a] L	C	H	HH	sed	sig.	Susceptibility[b] HS	LS	sed	sig
pre		9.80	9.00	9.07	8.85	0.42	ns	9.07	9.29	0.16	ns
post	0[c]	8.37	7.72	7.78	7.70	0.36	ns	7.75	8.03	0.14	ns
	1.5	7.21	7.00	7.18	7.14	0.25	ns	7.20	7.07	0.16	ns
	7.5	6.80	6.61	6.56	6.01	0.30	+	6.37	6.62	0.25	ns

[a] Averaged for all cows
[b] Averaged for all treatments
[c] Estimated from the DM content at 0 h with 10 ℓ solution added to the digesta

The pH of digesta increased during the hour before addition of solutions, and continued to increase after solution addition (Figure 5.4). The pH values of digesta significantly increased as electrolyte content of the solutions increased (Figure 5.4a), except at 7.5 h when there was no significant difference between treatments. HS cows maintained slightly higher pH values, by about 0.1 unit, than did LS cows (Figure 5.4b).

5.3.6 **Addition to digesta of KCl**

Effects of the addition of KCl solution on the osmolality and concentrations of Na and K in rumen fluid and on the quantity of digesta in the reticulo-rumen are presented for individual cows in Appendices 5a and 5b, respectively.

A high osmolality and concentration of K in rumen fluid sampled immediately after addition of the KCl solution for one cow indicated that the solution and digesta were poorly mixed in this cow at the time of sampling.

Addition of the treatment solution increased the concentration of K to 50-60 mmol/ℓ, with small effects on Na concentration. No differences between HS and LS cows in the rate of change of osmolality or concentrations of Na and K were apparent. Fluid losses over 7.5 h averaged 6.29 and 6.43 kg for HS and LS cows, respectively. On average there was a small net gain of fluid by LS cows over 0-1.5 h, compared to a loss for HS cows.

5.4 DISCUSSION

The effect of increasing the concentration of Na in rumen fluid through Na loading was to decrease the net fluid loss to the omasum, but it seems that decreased concentration of Na in rumen fluid was not associated with the higher fluid loss seen in LS cows compared to HS cows in this and earlier experiments.

Concentrations of Na and K in mixed saliva are higher and lower, respectively, than those in rumen fluid (Bailey 1916b) and the addition of solution C, containing concentrations of Na and K similar to those in parotid saliva, increased Na concentration and decreased K concentration in rumen fluid by 10 and 1 mmol/ℓ, respectively. The concentration of Na in rumen fluid remained elevated after addition of solution C, and K concentration decreased on all treatments over 7.5 h, probably due to the gradual increase in Na and decrease in K concentrations that occurs during fasting (Chapter 3.3.5).

Addition of water to the rumen decreased the osmolality and concentrations of Na and K in rumen fluid. Following water loading the osmolality increased at a rate of 4 mosmol/kg/h, similar to the rates of 4-7 mosmol/kg/h observed by Warner and Stacy (1965) following water loading in sheep. The increase in osmolality was due to an increase in concentration of Na rather than K. This has been found to result from the influx of saliva into the reticulo-rumen rather than from water or Na movements across the rumen wall (Warner & Stacy 1972b). Wilson (1963) reported that increasing the volume of digesta with rumen fluid or water decreased the parotid secretion in sheep, and attributed the effect to increased ruminal distension. However, the negative relationship found between salivary flow rate and the osmotic pressure of rumen fluid (Warner & Stacy 1977) or plasma (Carr & Titchen 1978) suggests that decreasing the osmolality of rumen fluid by water loading would increase the saliva flow rate and, therefore, increase the contribution of Na to reticulo-rumen contents. Any effect of the 10 ℓ water on distension would have been common to all treatments. That saliva entered the reticulo-rumen after addition of water alone was indicated by the increase over 7.5 h in the amount of Na in the digesta. The only evidence of Na entering the reticulo-rumen across the rumen wall has been found under conditions which are outside physiological limits, such as high K loading and osmotic pressures of 550 mosmol/kg (Warner & Stacy 1972b).

Similar net losses of fluid from the reticulo-rumen over 7.5 h occurred after additions of both water alone and solution C, although both net inflow and outflow tended to be higher on solution C than on water alone. If the inflow of saliva into the reticulo-rumen was higher after addition of water alone than after addition of solution C, resulting from the lower osmolality of digesta after addition of water, then the movement of water into the reticulo-rumen across the rumen wall must have contributed to the higher net inflow after addition of solution C.

Addition of solutions H and HH markedly increased the concentration of Na in rumen fluid and, in the absence of drinking water, 7.5 h was insufficient for the return of Na concentration to pre-treatment values or to values similar to those on solution C.

The net inflow of fluid to the reticulo-rumen was lower after addition of solution HH than of C, possibly due to an inhibition of parotid flow (Warner & Stacy 1977). An alternative explanation is that water moved out of the reticulo-rumen across the rumen wall, but this was unlikely since it has been shown that in the range of osmolality observed (325-425 mosmol/kg) water moves from the blood into the rumen (Dobson _et al_. 1970; Warner & Stacy 1972b). Elevation of K or Na concentration or osmotic pressure of digesta stimulates the absorption of Na from the reticulo-rumen (Stacy & Warner 1966, Warner & Stacy 1972b), thus the decrease in concentration and amount of Na in the digesta following addition of solution HH was due to absorption, rather than dilution and outflow, compared to solution C. Elevating the concentration of K to 50-60 mmol/ℓ with KCl had little effect on the concentration of Na in digesta. Although this K concentration was similar to that previously observed in Experiment 3, it may have been too low to result in increased absorption of Na.

The retention of fluid as the strength of electrolyte solution increased was due to a decrease in outflow rather than to an increase in net inflow to the reticulo-rumen, since the net inflow after addition of water alone and of solution HH were similar, although possibly of different origin. Absorption of Na from the rumen into the blood, elevating plasma Na concentration and osmolality (Warner & Stacy 1977) may have decreased the outflow of digesta from the reticulo-rumen after addition of solutions H and HH by causing a reduction in the frequency of reticular contractions (Carr & Titchen 1978). Alternatively, inhibition of abomasal emptying due to hyperosmolality of duodenal contents, as has been found to occur after infusion of hyperosmolal solutions into the duodenum of the milk-fed calf (Bell & Mostaghni 1975), may have slowed the rate of flow from the reticulo-rumen. Lack of any significant effect of contraction frequency on DM outflow is not unexpected since the changes in DM quantity were small. A 1% error in estimation of DM content of digesta would result in about a 10% error in DM quantity.

The pH of rumen fluid decreased after addition of water alone and increased after addition of the electrolyte solutions. However, the pH of rumen fluid was increasing during the hour before addition of solutions therefore the increase in pH after addition of solutions C, H and HH may not have been due solely to the solutions. Reducing the pH from 6.8 to 5.8 with HCl at 17 h after feeding has been found to increase the transfer of water across the rumen wall in both directions, with little effect on net flow (Willes et al. 1970). The pH on any of the treatments over 0-7.5 h did not exceed the pH at 7.5 h, suggesting that any effects of treatment on pH between 0-7.5 h were within physiological ranges.

The loss of fluid from the reticulo-rumen was greater for LS than for HS cows irrespective of the Na concentration over the range measured, and was apparently not the result of an increase in quantity of fluid in the reticulo-rumen of LS cows after addition of treatments. The lack of a relationship between the quantity of fluid in the reticulo-rumen and subsequent loss is not in agreement with previous results (Experiments 3, 4, 5) possibly because the range between cows in weight of digesta was less than half those found in previous experiments, or because of an effect on the relationship due to dilution of digesta with water. Simpson (1969) observed that adding solutes to the rumen in a large volume of water resulted in a slower excretion of water and solutes in urine, but an increase in the total excreted, than when the same solute load was added in a small volume of water. It was presumed that the higher volume of water resulted in an increase in outflow of the water and solutes to more distal regions of the gut.

The range of treatments and their resulting osmotic conditions in the reticulo-rumen should have invoked responses involving regulation of electrolyte absorption and fluid movements into and out of the reticulo-rumen. Given that the techniques used only measured the net effects of movements of electrolytes and water, LS and HS cows only differed in net flow of fluid out of the reticulo-rumen. Large electrolyte loads delivered rapidly to the reticulo-rumen may have different effects on digesta composition and outflow rate compared to chronic high levels of Na or K intake or to changes in the ratio of K and Na in the diet. The effects of dietary K:Na ratio on digesta characteristics are investigated in the final experiment.

CHAPTER 6

THE EFFECT OF Na AND K INTAKES ON THE QUANTITY AND COMPOSITION OF DIGESTA IN THE RETICULO-RUMEN AND ON MINERAL METABOLISM OF COWS DIFFERING IN SUSCEPTIBILITY TO BLOAT

6.1 INTRODUCTION

Pasture diets offered to dairy cows in New Zealand often contain quantities of K in excess of the cow's requirements, but are of marginal or low Na content (Smith & Middleton 1978). High K, low Na herbages have been implicated in causing bloat, compared to herbages of low K:Na ratio (Turner 1981), but the mechanism by which Na and K or the K:Na ratio may affect bloat has not been established. Variations in intake of Na and K can have marked effects on electrolyte and fluid absorption from digesta, fluid outflow from the reticulo-rumen, and metabolism of Na and K by the kidney (Warner & Stacy 1972a).

The change in diet from hay to pasture which resulted in a difference between susceptibility groups in the quantity of digesta in the reticulo-rumen in Experiment 3 also involved a change in intake of Na and K. The extent to which the intakes of Na and K were responsible for the observed difference in the quantity of digesta is not known. This chapter describes an experiment (Experiment 8) which investigated the effects on the quantity and composition of digesta in the reticulo-rumen of changes in the K:Na ratio of the diet. Other measurements included the concentrations of Na and K in saliva, blood, urine and faeces to assess whether cows which differed in susceptibility also differed in mineral metabolism.

6.2 MATERIALS AND METHODS

6.2.1 Herbage preparation

One half of each of two areas of pasture (pastures A and B), totalling 2.4 ha, were manipulated to produce herbage of high K:Na ratio (HR) and the other, herbage of low K:Na ratio (LR). From November 1984 until March 1985 the pastures were managed as follows:

HR - These areas were grazed on 3 occasions. The second and third grazings were followed by applications of 50 and 100 kg KCl per ha, respectively.

LR - Herbage was cut and removed on 2 occasions, each followed by an application of 100 kg NaCl per ha.

Both areas received 50 kg N per ha (as urea) in December 1984. Areas were irrigated by sprinkler when the soil moisture deficit was greater than 35 mm. The deficit was calculated from daily open-pan evaporimeter measurements at the Ruakura Climatological Station and the daily rainfall measured at the site. Irrigation was also used to prevent leaf burn following application of fertiliser.

Pastures A and B differed in Na and K contents and in K:Na ratio on each of the HR and LR areas when sampled (in duplicate) prior to the feeding experiment (Table 6.1), although the ratio was 4-5 times higher in HR herbage than LR herbage for both pastures. Botanical composition (Table 6.1) determined from dissection of the samples indicated the pastures consisted predominantly of perennial ryegrass and white clover, with contributions of paspalum (*Paspalum dilatatum* Poir.) to LR herbage in pasture B, and prairie grass to HR herbage in pasture A. To avoid changing pasture type during the feeding experiment, each cow was offered 50% of her DM intake from each of pastures A and B.

Table 6.1 The concentrations in herbage of Na and K (g/100 g DM) and the K:Na ratio, and the botanical composition (% DM) of areas of high (HR) and low (LR) K:Na ratio from 2 pastures (A, B) after addition of KCl or NaCl fertiliser.

Pasture	A		B	
Herbage ratio	HR	LR	HR	LR
Na	0.09	0.35	0.20	0.66
K	4.15	3.48	3.05	2.19
K:Na	46	10	15	3
Botanical composition				
Ryegrass	51	54	66	51
White clover	19	32	17	15
Other grasses	12	3	10	24
Weeds	14	6	6	5
Dead material	4	5	1	5

6.2.2 Cows

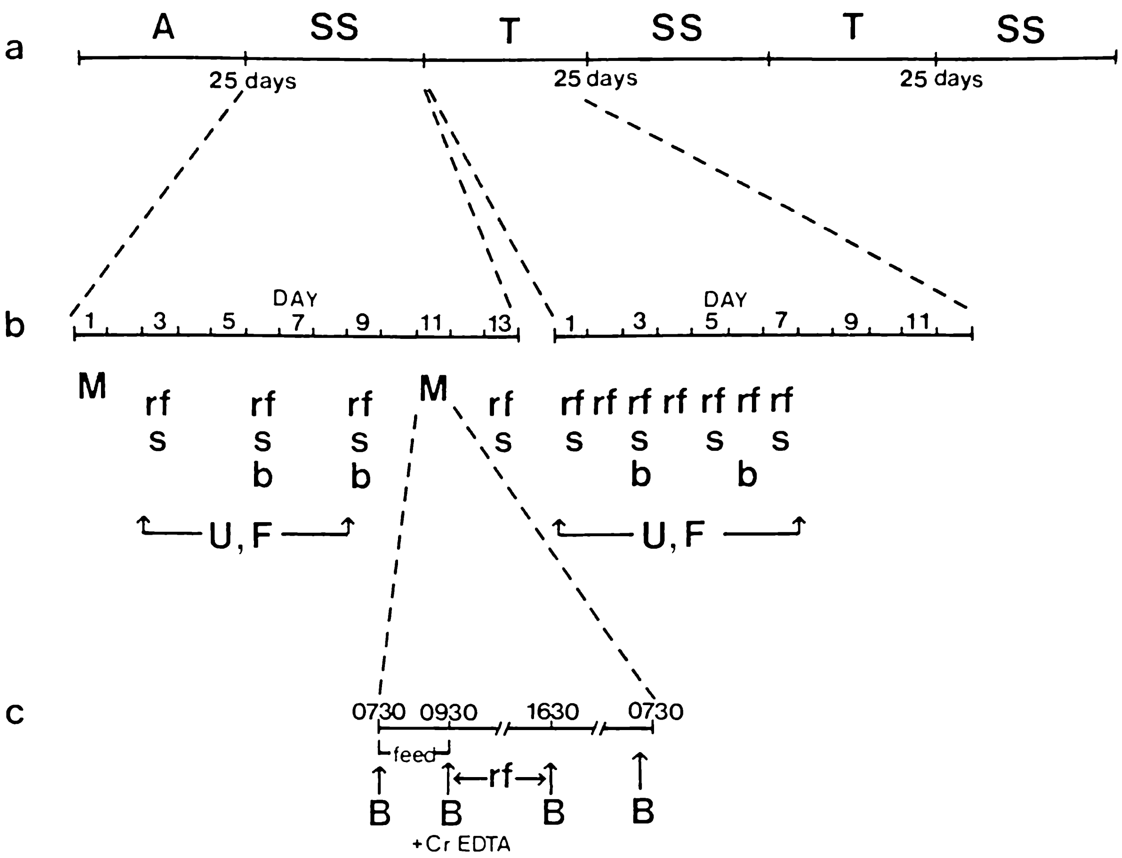
The cows were those used in Experiment 7. They were prevented from cycling in oestrus as described in Chapter 5.2.1.

The cows were housed in indoor feeding stalls and yards, as in Chapter 3.2.1, except during collection of faeces and urine when they were housed in digestibility stalls (Chapter 2.2.1).

6.2.3 Feeding, experimental design and measurements

Feeding The diets were offered at a maintenance feeding level as described in Chapter 3.2.2 using live weights prior to the experiment. Food was offered for 2 h each day and water offered to appetite at the end of the feeding period. The cows were managed in 2 groups (1 HS, 1 LS per diet) which differed in feeding and measurements by 1 h. Food was offered at 0730 and 0830 h.

The pastures were cut daily at 1530 h using a reel mower, gathered by hand, and spread over a concrete floor to prevent heating of the herbage during the delay until feeding.



KEY	A	Adaptation (no measurements)
	SS	Steady state
	T	Transition
	M	Digesta measurements
	B	Bailing
	U	Urine collection
	F	Faeces collection
	rf	Rumen fluid sampling
	s	Saliva sampling
	b	Blood sampling

Figure 6.1 The schedule of measurements for Experiment 8.

The weight of herbage offered was based on the DM contents obtained by oven-drying duplicate 100 g samples overnight.

Experimental design The feeding experiment consisted of 3, 25-day periods (Figure 6.1a). In each period two HS and 2 LS cows were offered the HR diet and the remaining cows offered the LR diet. The cows changed diets between periods so that 4 cows were offered the diets in the order of HR, LR, HR and the remaining cows LR, HR, LR. Each 25-day period consisted of 12 days adaptation, or transition, followed by a 13-day steady state, and measurements were made during each of these with the exception of the first adaptation period.

The final steady state period was not completed because of a shortage of food.

Steady state measurements

Digesta Digesta in the reticulum and rumen on days 1 and 11 was removed from each cow, weighed, sampled for DM content and replaced immediately before feeding and at 0, 7 and 21.5 h after feeding (Figure 6.1b,c). Cr EDTA was added to digesta removed at 0 h. Rumen fluid was sampled from digesta removed and from the reticulo-rumen at 1-2 hourly intervals between 0 and 7 h, and analysed for osmolality and concentrations of Na, K and Cr.

Rumen fluid The osmolality and concentrations of Na and K were measured in rumen fluid sampled before and after feeding on days 3, 6, 9 and 13 (Figure 6.1b).

Saliva The concentrations of Na and K were measured in saliva (sampled from between the cheek and molars) before and after feeding on days 3, 6, 9 and 13 (Figure 6.1b).

Blood Blood (10 ml) was withdrawn from the jugular vein into heparinised vacutainers before and after feeding on days 6 and 9, for measurement of PCV, plasma osmolality and concentrations of Na and K.

Faeces and urine Complete collections of faeces and urine were made during days 3-8 with daily aliquot samples of each for each cow bulked over the 6-day period (Figure 6.1b). Bulked samples were analysed for concentrations of Na, K, Mg, Ca, P and also in the case of faeces for N and energy.

Transition measurements

Sampling of rumen fluid, saliva and blood both before and after feeding, and the collection of faeces and urine were continued as follows (Figure 6.1b):

Rumen fluid was sampled on days 1-7 following the change in diet.

Saliva was sampled on days 1, 3, 5 and 7 following the change in diet.

Blood was sampled on days 3 and 6 following the change in diet.

Faeces and urine were collected, weighed and subsampled every 24 h for each cow for 7 days following the change in diet, and the daily samples analysed for concentrations of minerals.

6.2.4 Techniques, sampling and analytical procedures

Foods and residues Two subsamples of each food type were taken daily as food boxes were filled. One sample was frozen and the second was analysed for DM content. Frozen samples were bulked within food type over 4-6 day periods for subsequent analysis. Any residues were weighed, sampled and analysed for DM content and the dried material was retained for mineral analysis.

Procedures for the analysis of the DM, nitrogen, energy, water-soluble sugar, MAD fibre, and mineral concentrations in foods were described in Chapters 2.2.5 and 3.2.4.

Digesta, rumen fluid, saliva and blood Techniques for digesta removal, sampling and Cr EDTA addition and for collection and preparation of rumen fluid, saliva and blood samples were described in Chapter 3.2.3. Procedures for analysis of the DM content of digesta, osmolality and concentrations of Na and K in rumen fluid, saliva and plasma and packed cell volume of blood were described in Chapter 3.2.3 and 3.2.4.

Faeces and urine Collection and preparation of faeces and urine samples and the methods of analysis for DM, nitrogen, energy and mineral concentrations in faeces and mineral concentrations in urine were described in Chapter 2.2.4 and 2.2.5. The volume of urine was used to estimate its water content and was determined from its weight and specific gravity, obtained by weighing duplicate 100 ml subsamples of each urine sample collected.

Leakage of rumen fluid from the fistula proved to be a problem (see later). When the cows were in digestibility stalls, an estimate of the quantity of fluid which leaked out of the fistula onto the floor was made by drainage of the fluid through a small hole in the centre of the floor of the digestibility stall.

6.2.5 Calculations and statistical analysis

Digesta losses were calculated as described in Chapter 3.2.5, using the time intervals of a 150 minute feeding period and post-feeding periods of 420 minutes (0-7 h) and 870 minutes (7-21.5 h), but without using regression analysis to adjust for samples removed.

The rumen fluid dilution rate and net inflow to the reticulo-rumen and outflow to the omasum were estimated as described in Chapter 3.2.5.

The data were analysed for differences between susceptibility groups and diets using analysis of variance models provided by Genstat V.

Table 6.4 Intakes (kg/cow) on digesta measurement days and the quantities (kg) of fluid, DM and total digesta removed from the reticulo-rumen before and at 0, 7 and 21.5 h after feeding for HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

Time relative to feeding (h)	HR		LR									
	HS	LS	HS	LS	sed^a	sig.	LR-HR^b	sed	sig.	LS-HS^c	sed	sig.
Fluid												
Intake	25.23	27.45	26.19	25.64	1.05	+	-0.43	0.63	ns	0.84	0.84	ns
Digesta pre-feed	35.75	29.59	38.63	31.36	2.77	ns	2.33	1.00	+	-6.72	2.58	*
post-feed 0	57.04	55.30	61.04	57.19	2.68	ns	2.95	1.34	+	-2.80	2.33	ns
7	48.42	42.30	51.38	44.12	3.80	ns	2.39	1.34	ns	-6.69	3.56	ns
21.5	35.41	29.71	38.50	31.95	3.17	ns	2.67	1.14	+	-6.13	2.94	+
DM												
Intake	4.43	4.69	4.25	4.47	0.13	ns	-0.20	0.01	***	0.24	0.13	+
Digesta pre-feed	2.29	2.11	2.42	2.19	0.22	ns	0.11	0.09	ns	-0.21	0.20	ns
post-feed 0	5.98	6.31	6.55	6.44	0.30	ns	0.35	0.12	*	0.11	0.27	ns
7	3.78	3.80	4.43	4.24	0.25	ns	0.55	0.11	**	-0.09	0.22	ns
21.5	2.09	1.95	2.17	2.10	0.24	ns	0.12	0.06	ns	-0.11	0.23	ns
Total												
Intake	29.66	32.14	30.44	30.11	1.11	ns	-0.62	0.63	ns	1.09	0.91	ns
Digesta pre-feed	38.04	31.70	41.05	33.55	2.96	ns	2.43	1.07	+	-6.92	2.76	*
post-feed 0	63.02	61.61	67.59	63.63	2.94	ns	3.29	1.39	+	-2.69	2.59	ns
7	52.20	46.10	55.81	48.36	3.93	ns	2.94	1.33	+	-6.78	3.70	ns
21.5	37.50	31.66	40.67	34.05	3.38	ns	2.79	1.17	+	-6.24	3.17	+

a,b,c see Table 6.3

Table 6.3 Daily intakes of DM and water (kg/cow) and of minerals (g/cow) by HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

	HR		LR									
	HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
DM	4.40	4.49	4.37	4.60	0.12	ns	0.04	0.02	ns	0.16	0.12	ns
Water F[d]	20.6	21.3	18.4	19.2	0.5	ns	-2.2	0.1	***	0.8	0.5	ns
D	3.3	6.0	5.4	6.7	1.6	+	1.4	0.3	**	2.0	1.6	ns
T	23.9	27.3	23.8	25.9	1.5	ns	-0.8	0.4	ns	2.8	1.5	ns
Na	9.0	9.4	20.9	22.2	0.4	ns	12.4	0.2	***	0.9	0.5	ns
K	172.5	180.3	138.3	145.1	4.3	ns	-34.7	1.0	***	7.3	4.2	ns
Ca	33.3	34.7	32.8	34.4	0.9	ns	-0.4	0.1	**	1.5	0.9	ns
Mg	9.3	9.7	9.6	10.1	0.3	ns	0.4	0.04	***	0.5	0.3	ns
P	12.4	12.9	12.9	13.6	0.3	ns	0.6	0.1	***	0.6	0.3	ns

[a] The standard error of the difference for the interaction between susceptibility group and diet

[b] The difference between the diets (LR minus HR) when averaged for all cows

[c] The difference between susceptibility groups (LS minus HS) when averaged for both diets

[d] F = feed; D = drunk; T = total

Table 6.2 Energy content (MJ/kg DM) and concentrations (g/100 g DM) of MAD fibre, water-soluble sugar, nitrogen (N) and minerals in diets of high (HR) and low (LR) K:Na ratio.

	HR	se[a]	LR	se
Energy	18.93	0.12	18.57	0.09
MAD fibre	25.09	0.68	27.62	0.69
Sugar	14.37	0.87	13.38	0.70
N	3.22	0.11	3.02	0.07
Na	0.21	0.01	0.48	0.01
K	3.97	0.06	3.46	0.09
Ca	0.76	0.02	0.75	0.03
Mg	0.21	0.01	0.22	0.01
P	0.29	0.01	0.30	0.01

[a] Standard error for the mean of 10 samples taken over the experimental period

6.3 RESULTS

6.3.1 **Diet composition and intake data**

The concentrations of Na and K in the LR diet were, respectively, 2.29 and 0.87 times those in the HR diet (Table 6.2). Intakes of Na averaged 9.2 and 21.6 g (400 and 937 mmoles) and those of K, 176 and 142 g (4.501 and 3.632 moles) for the HR and LR diets, respectively (Table 6.3). Intakes of Ca were slightly lower and those of Mg and P slightly higher on the LR diet compared to the HR diet. There were no differences between susceptibility groups in the intakes of minerals.

DM intakes averaged 4.39 and 4.55 kg/cow for HS and LS cows, respectively (Table 6.3), or 47 and 46 g/kg $LW^{0.75}$, respectively. The quantity of water consumed in the herbage was significantly lower when the diet was LR than when it was HR, and this was compensated for by a difference in water drunk so that there was no effect of diet on the total water consumption (Table 6.3). There was no difference between susceptibility groups in water consumption, although there was an interaction ($P<0.10$) between diet and susceptibility group, the difference due to diet in amount drunk being greater for HS than for LS cows.

6.3.2 **Quantity of digesta in the reticulo-rumen**

Intakes of DM and fluid on digesta measurement days did not differ between susceptibility groups or between diets (Table 6.4), except that LS cows consumed slightly less water when offered LR and more when offered HR than HS cows.

LS cows had significantly less fluid, but not DM, in the reticulo-rumen before feeding (pre-feed and 21.5 h estimates) than did HS cows on both the HR and LR diets (Table 6.4). After feeding, HS and LS cows had similar quantities of fluid and DM at 0 h, and of DM at 7 h, but LS cows had 6.69 kg less fluid than HS cows at 7 h.

Table 6.6 The percentage of DM in the digesta of HS and LS cows offered diets of high (HR) and low (LR) K:Na ratip

Time of sampling (h)	HR		LR									
	HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
Pre-feed	6.03	6.71	5.87	6.66	0.37	ns	-0.11	0.17	ns	0.74	0.74	+
Post-feed 0	9.51	10.25	9.70	10.13	0.24	ns	0.04	0.21	ns	0.59	0.12	**
7	7.32	8.24	7.99	8.86	0.56	ns	0.65	0.31	+	0.90	0.47	ns
21.5	5.57	6.20	5.33	6.23	0.35	ns	-0.11	0.18	ns	0.77	0.30	*

a,b,c, see Table 6.3

Table 6.5 Calculated losses (kg) of fluid, DM and total digesta from the reticulo-rumen during feeding and over 7 and 21.5 h after feeding for HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

	Time (h)	HR		LR									
		HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
Fluid													
Feeding		1.14	-0.63	1.32	-2.42	1.10	**	-0.81	0.24	*	-2.76	1.08	*
Post-feed	0-7	6.49	11.36	7.41	11.38	2.18	ns	0.46	0.46	ns	4.42	2.14	+
	7-21.5	10.85	10.57	10.78	10.15	1.65	ns	-0.25	0.80	ns	-0.46	1.44	ns
	0-21.5	17.34	21.93	18.19	21.53	1.69	ns	0.23	0.76	ns	3.97	1.51	*
DM													
Feeding		0.59	0.35	-0.04	0.08	0.20	+	-0.45	0.09	**	-0.06	0.18	ns
Post-feed	0-7	1.84	2.17	1.78	1.87	0.20	ns	-0.18	0.15	ns	0.21	0.13	ns
	7-21.5	1.55	1.70	2.12	1.98	0.20	ns	0.43	0.11	+	0.01	0.17	ns
	0-21.5	3.39	3.87	3.90	3.85	0.16	*	0.25	0.08	*	0.22	0.13	ns
Total													
Feeding		1.73	-0.28	1.28	-2.34	1.13	**	-1.26	0.19	***	-2.81	1.18	*
Post-feed	0-7	8.33	13.53	9.19	13.25	2.09	ns	0.30	0.47	ns	4.64	2.04	+
	7-21.5	12.40	12.27	12.90	12.14	1.68	ns	0.19	0.84	ns	-0.45	1.46	ns
	0-21.5	20.73	25.80	22.09	25.38	1.72	ns	0.47	0.73	ns	4.18	1.55	*

a,b,c, see Table 6.3

Although this difference in fluid was not significant, it is of similar magnitude to the difference between susceptibility groups in quantity of fluid before feeding (6.72 kg) or at 21.5 h (6.13 kg), indicating that the difference between susceptibility groups in digesta quantity at 21.5 h had arisen within 7 h of feeding.

The quantity of fluid, but not DM, in the reticulo-rumen both before and after feeding was higher on the LR diet than on the HR diet. The difference due to diet in the quantity of fluid (2-3 kg) was less than half the difference obtained between susceptibility groups.

LS cows showed a net gain of fluid during feeding, compared to a net loss for HS cows (Table 6.5). LS cows lost more fluid than HS cows during 0-7 h after feeding, but not subsequently. DM losses were similar for both susceptibility groups during and after feeding.

The net losses of fluid and DM during feeding were greater in cows when offered the HR diet than when offered the LR diet. There were no significant effects of diet on losses after feeding, except that DM losses over 7-21.5 h and 0-21.5 h were higher on the LR diet than on the HR diet (Table 6.5). There was an interaction between diet and susceptibility group in fluid loss during feeding, the difference between susceptibility groups being greater when offered the LR diet than when offered the HR diet.

Quantities of digesta at 21.5 h were similar to those measured before feeding (Table 6.4) but calculated losses over 24 h were about 7 kg lower than the quantities of fluid and DM consumed. Part of this difference can be accounted for by digesta removed in samples at the 4 digesta bailings.

The DM content of digesta was higher for LS cows than for HS cows before feeding and at 0 and 21.5 h after feeding (Table 6.6). DM content at 7 h was higher in digesta of cows offered the LR than the HR diet but there were no effects of diet at the other sample times. There were no interactions between diet and susceptibility group.

6.3.3 Rumen fluid dilution rate

The dilution rate over 7 h after feeding was higher for LS than for HS cows on both diets, but there was no significant difference between susceptibility groups in the net fluid inflow to the reticulo-rumen (Table 6.7). Outflow to the omasum was higher for LS than for HS cows. Both net inflow and outflow were significantly higher when cows were offered the LR diet than when offered the HR diet. The effects of diet on inflow and outflow were smaller than the differences between susceptibility groups, but were significant because of the relatively low standard errors of the differences between diet means compared to those for susceptibility group means.

Table 6.7 Rumen fluid dilution rates (D, %/h) and net inflow to the reticulo-rumen (I, ℓ/h) and outflow to the omasum (O, ℓ/h) for HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

	HR		LR									
	HS	LS	HS	LS	sed^a	sig.	LR-HR^b	sed	sig.	LS-HS^c	sed	sig.
D	6.87	9.76	7.90	9.97	1.32	ns	0.62	0.53	ns	2.48	1.21	+
I	3.52	4.71	4.30	4.92	0.60	ns	0.50	0.17	*	0.91	0.58	ns
O	4.49	6.41	5.40	6.62	0.80	ns	0.56	0.21	*	1.57	0.77	+

a,b,c see Table 6.3

6.3.4 Composition of rumen fluid on digesta measurement days

Osmolality Osmolality values of rumen fluid were significantly higher after feeding when cows were offered the HR diet than when offered the LR diet (Figure 6.2), averaging 302 and 295 mosmol/kg,

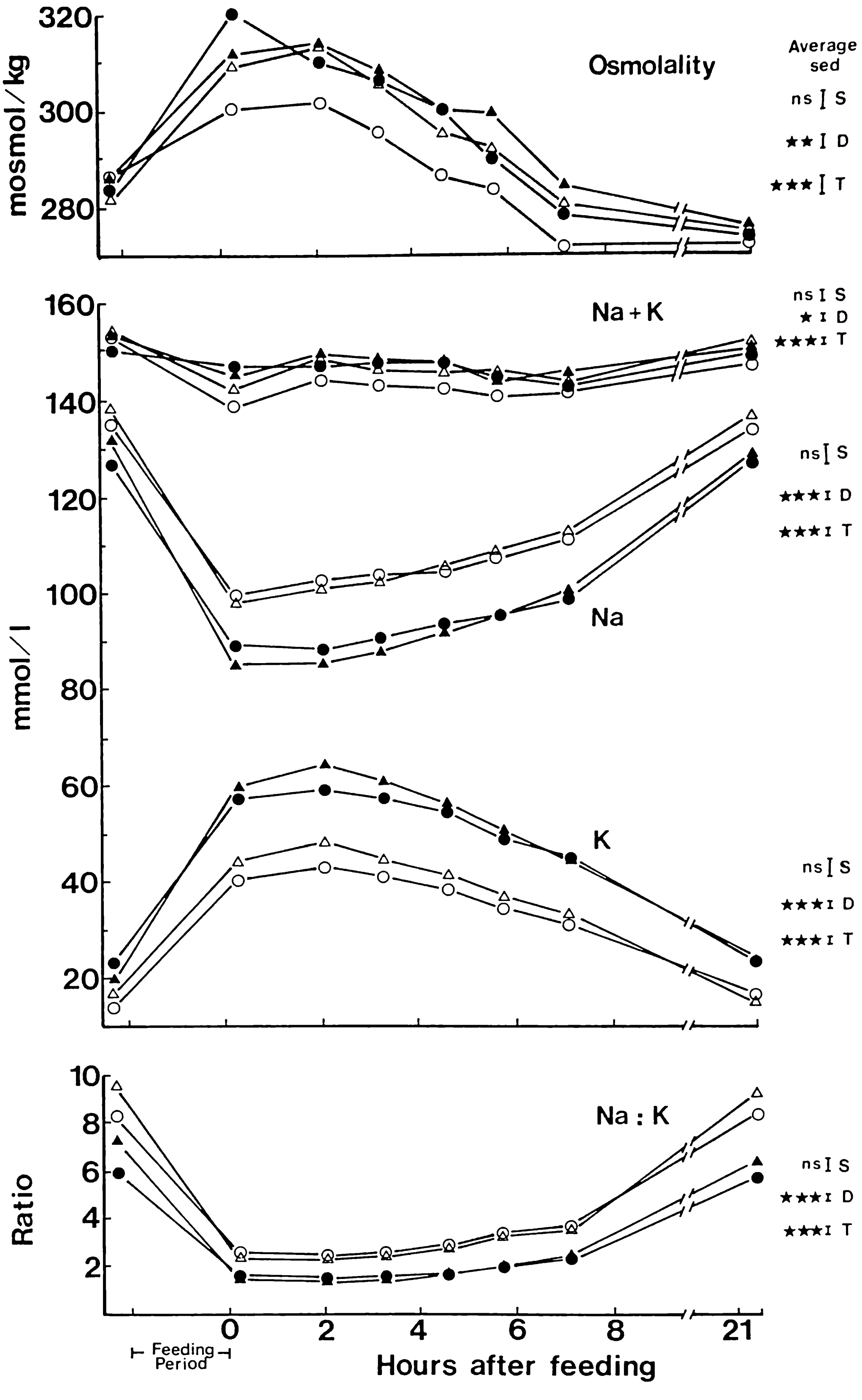


Figure 6.2 Effects of time relative to feeding on osmolality, concentrations of Na, K, Na + K and Na:K ratio in rumen fluid from HS (●,○) and LS (▲,△) cows offered diets of high (●,▲) and low (○,△) K:Na ratio. Sed values are for comparisons between susceptibility groups averaged for diets and times (S), between diets averaged for susceptibility groups and times (D) and between times of sampling averaged for susceptibility groups and diets (T).

respectively, over the 7 h post-feeding period. There were no significant differences between susceptibility groups, values averaging 295 and 301 mosmol/kg for HS and LS cows, respectively. There was no interaction between diet and susceptibility group.

Osmolality differed significantly between sample times, reaching a maximum within 2 h of feeding.

Na and K Concentrations of Na were significantly higher and those of K significantly lower at all sample times when cows were offered the LR diet than when offered the HR diet (Figure 6.2). Average values over the post-feeding period for Na were 92.0 and 104.8 mmol/ℓ for HR and LR diets, respectively. Corresponding values for K were 54.9 and 39.2 mmol/ℓ.

The Na + K concentration of rumen fluid was slightly but significantly higher after feeding when cows were offered the HR diet than when offered the LR diet, values averaging 146.9 and 144.0 mmol/ℓ, respectively.

The Na:K ratio was significantly higher in rumen fluid from cows when offered the LR diet than when offered the HR diet, with values averaging 2.76 and 1.76, respectively, during the post-feeding period. There were no significant differences between susceptibility groups in concentrations of Na, K, Na + K or the Na:K ratio, but values differed between sample times.

6.3.5 Steady state comparisons for rumen fluid, saliva and blood

Diet effects Offering the LR diet increased the concentration of Na and decreased the concentration of K in rumen fluid both before and after feeding, compared to offering the HR diet (Table 6.8, Figure 6.3). The magnitude of the difference between diets in concentration of Na before feeding was similar to that in concentration of K, therefore the sum of Na and K was not affected by diet. After

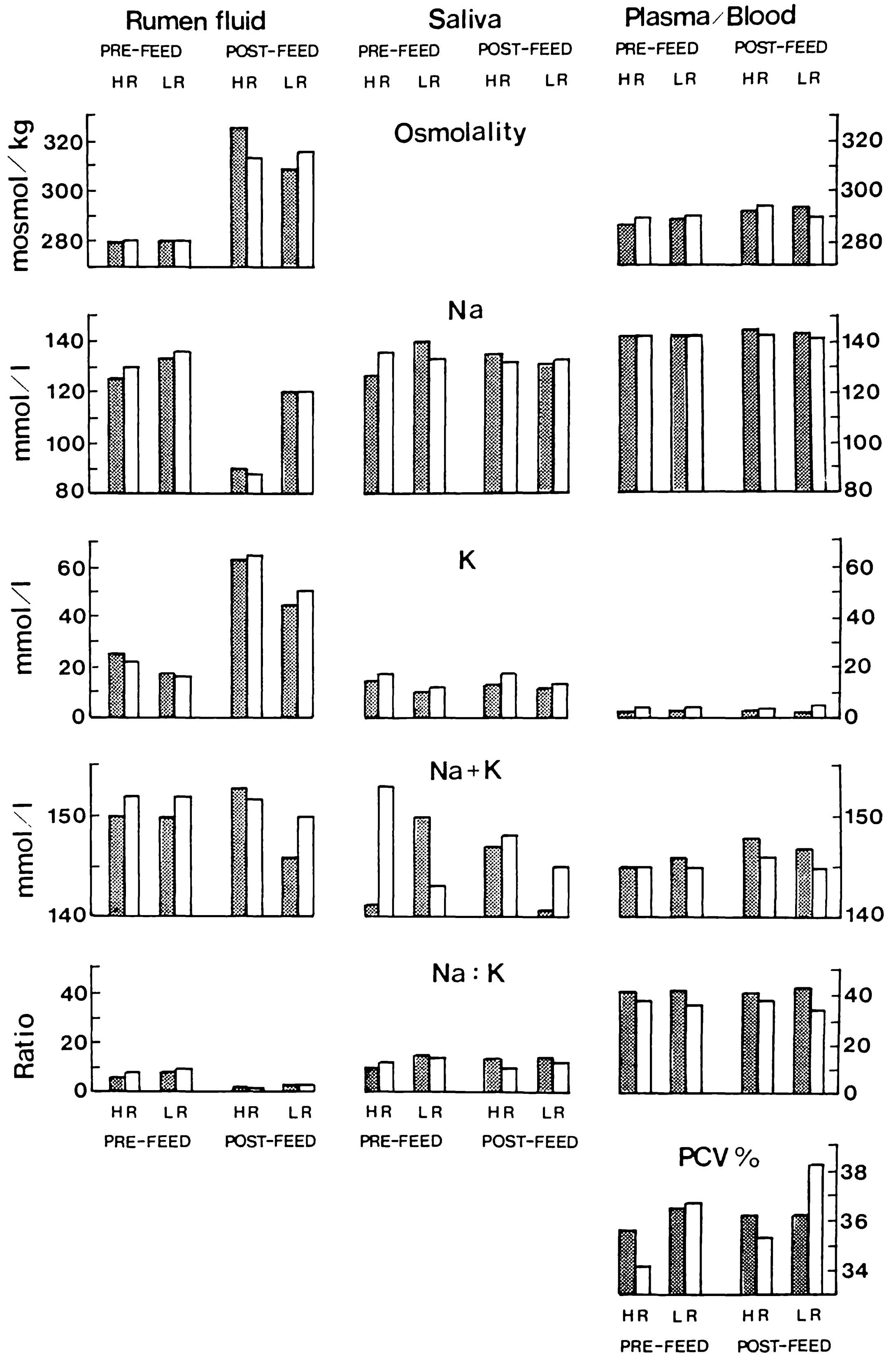


Figure 6.3 The composition of rumen fluid, saliva and blood from HS (▨) and LS (□) cows before and after feeding on diets of high (HR) and low (LR) K:Na ratio.

Table 6.8 A summary of the main effects of diet and susceptibility group on rumen fluid, saliva and blood composition before and after feeding.

		Diet			Susceptibility		
		LR-HR	sed	sig.	LS-HS	sed	sig.
Rumen fluid							
pre-feed	Osmol	0.3	0.8	ns	0.9	2.7	ns
	Na	7.0	1.6	**	4.0	3.0	ns
	K	-6.9	1.7	**	-1.9	2.8	ns
	Na + K	0.1	0.7	ns	2.0	1.3	ns
	Na:K	2.3	0.4	**	1.1	1.0	ns
post-feed	Osmol	-7.8	3.8	+	-2.1	6.8	ns
	Na	11.9	1.8	**	-2.0	2.3	ns
	K	-16.5	1.4	***	4.0	2.5	ns
	Na + K	-4.6	1.4	*	2.0	3.4	ns
	Na:K	0.7	0.1	***	-0.2	0.1	ns
Saliva							
pre-feed	Na	4.6	2.9	ns	1.3	7.9	ns
	K	-5.3	1.7	*	2.0	2.1	ns
	Na + K	-0.7	3.2	ns	3.2	6.0	ns
	Na:K	3.8	1.5	+	-0.1	2.2	ns
post-feed	Na	-1.6	8.2	ns	-0.6	6.1	ns
	K	-2.8	1.2	+	3.7	2.8	ns
	Na + K	-4.5	7.6	ns	3.2	5.1	ns
	Na:K	2.2	2.4	ns	-2.4	2.7	ns
plasma/blood							
pre-feed	Osmol	1.8	-		1.0	-	
	Na	0.8	1.0	ns	-0.4	0.8	ns
	K	0.02	0.07	ns	0.4	0.15	*
	Na + K	0.8	1.0	ns	0.1	0.6	ns
	Na:K	0.4	1.4	ns	-4.7	1.9	*
	PCV	1.8	0.6	*	-1.8	1.4	ns
post-feed	Osmol	-0.6	-		-0.8	-	
	Na	-1.5	1.1	ns	-2.5	1.1	+
	K	0.1	0.1	ns	0.4	0.17	*
	Na + K	-1.4	1.1	ns	-0.1	1.1	ns
	Na:K	0.3	1.6	ns	-5.8	1.7	*
	PCV	1.5	0.7	+	0.5	1.7	ns

feeding, the Na + K concentration was higher on the HR diet than on the LR diet. Osmolality of rumen fluid before feeding was not affected by diet, but was higher after feeding on the HR diet than on the LR diet.

Concentration of Na in saliva before or after feeding was not affected by diet, but the concentration of K was higher in saliva from cows offered the HR diet than the LR diet both before and after feeding. The Na:K ratio was lower in saliva taken before feeding from cows when offered the HR diet than when offered the LR diet.

Concentrations of Na and K in plasma were not affected by diet, either before or after feeding. Half (16/32) of the steady state plasma samples were not analysed for osmolality due to problems with the osmometer, therefore statistical analysis of osmolality data is incomplete. The PCV was significantly higher in cows when offered the LR diet than when offered the HR diet, both before and after feeding.

Susceptibility effects There were no significant differences between susceptibility groups in rumen fluid or saliva parameters either before or after feeding when averaged for both diets (Table 6.8, Figure 6.3). There was a significant interaction ($P<0.05$) between diet and susceptibility group in the concentration of Na in saliva before feeding, being higher on the LR diet than on the HR diet for HS cows but not for LS cows.

The concentration of K in plasma was significantly higher and the Na:K ratio lower for LS cows than for HS cows, both before and after feeding on both diets (Table 6.8, Figure 6.3). Plasma Na concentration was lower for LS cows than for HS cows after feeding. There were significant interactions ($P<0.10$) between diet and susceptibility group in plasma K and blood PCV after feeding. LS cows increased but HS cows decreased their plasma K concentration on the LR diet compared to the HR diet. The PCV of HS cows was unaffected by diet but LS cows had a higher PCV on the LR diet than on the HR diet.

Pre-feed versus post-feed The concentration of Na and the Na:K ratio in rumen fluid significantly decreased ($P<0.001$) and the K concentration and osmolality significantly increased ($P<0.001$) during feeding. The increase in K concentration and osmolality were greater ($P<0.01$, $P<0.10$, respectively) when the cows were offered the HR diet than when offered the LR diet, and the changes in concentration of Na and K over feeding were greater for LS cows than for HS cows ($P<0.05$).

The composition of saliva was not significantly different before and after feeding.

Feeding significantly increased concentrations of Na and Na + K in plasma ($P<0.05$) and possibly osmolality, without affecting PCV or K concentration. The response in Na and Na + K concentrations to feeding was greater for HS than for LS cows ($P<0.05$).

6.3.6 Steady state comparisons for mineral outputs in urine and faeces, and for digestibility of the diets

Urine and faeces

Leakage of fluid from the fistula was a major problem when quantitatively describing water, Na and K outputs in urine and faeces, particularly those of Na. Measurement of the weight of fluid and the concentrations of Na and K in rumen fluid lost through leakage did not begin until the first transition period, therefore leakage data are only available for the second of the 2 steady state periods. For some cows the quantity of leaked fluid increased with increasing time spent in the digestibility stalls, apparently because they spent more time lying down, which was when most leakage occurred. On several occasions the fluid collected also contained urine if problems with urine collection occurred. These contaminated collections could be identified by the relatively high concentrations of K in the collected fluid.

The average daily weights (± standard error) of leaked fluid over 20 days (one steady state and two transition periods) were 0.96±0.57 kg and 2.90±0.77 kg/cow for HS and LS cows, respectively. Individual daily weights ranged from 0 to 7.85 kg. The average daily losses of Na over all days (including urine contaminated samples) were 2.7±1.6 g and 8.0±2.2 g for HS and LS cows, respectively. Corresponding losses of K were 5.6±3.4 g and 6.7±2.2 g. Individual daily losses ranged from 0-23.7 g Na and 0-61.7 g K. The maximum loss of Na in a non-urine contaminated collection was 21.7 g, this being from a cow on the HR diet and therefore equivalent to about 2.4 times her daily Na intake. The maximum loss of K in a non-urine contaminated collection was 15.2 g, and the highest loss as a percent of daily K intake was about 10%, or 13.6 g K.

Despite the loss of fluid and electrolytes through leakage, the losses of water and minerals in faeces and urine during the steady states have been analysed and the data presented. Quantities of water consumed and excreted (Table 6.9) indicated there was no difference between susceptibility groups or effect of diet on the quantity of water excreted in faeces or urine, except LS cows excreted more water in urine than did HS cows on both diets. The quantity of leaked fluid collected from the fistula during the second steady state period (first steady state data not available) averaged 1.26±0.79 kg and 2.35±0.89 kg for HS and LS cows, respectively (this includes 8 instances of urine contamination), which accounts in part for the proportion of water consumed which was not accounted for in faeces and urine.

Table 6.10 Daily mineral intake (I, g/cow) and output, expressed as a percent of intake, in faeces (F), urine (U), or retained and leaked (R+L) by HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

	HR		LR									
	HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
Na												
I	8.8	9.0	19.6	20.9	0.4	ns	11.4	0.2	***	0.8	0.3	+
F	16.7	18.3	15.9	6.6	8.9	ns	-6.3	6.7	ns	-3.9	5.8	ns
U	39.5	39.0	43.1	40.0	19.0	ns	2.3	6.7	ns	-1.8	17.8	ns
R+L	43.8	42.7	41.0	53.4	20.8	ns	4.1	10.3	ns	5.6	18.1	ns
K												
I	172.4	180.4	145.3	149.5	5.1	ns	-29.0	1.8	***	6.1	4.7	ns
F	7.0	4.6	10.3	5.7	1.9	ns	2.2	1.7	ns	-3.5	0.8	**
U	83.4	79.9	72.6	82.3	7.3	ns	-4.2	3.6	ns	3.1	6.3	ns
R+L	9.6	15.5	17.1	12.0	8.2	ns	2.0	5.0	ns	0.4	6.4	ns
Mg												
I	9.3	9.6	9.6	10.0	0.3	ns	0.4	0.04	***	0.4	0.3	ns
F	79.8	90.6	84.9	94.9	7.8	ns	4.7	3.2	ns	10.4	7.1	ns
U	12.0	10.5	12.5	12.5	1.7	ns	1.3	0.9	ns	-0.8	1.4	ns
R+L	8.2	-1.1	2.6	-7.4	7.6	ns	-6.0	3.1	ns	-9.7	7.0	ns
Ca												
I	32.1	33.2	34.2	35.6	1.0	ns	2.3	0.1	***	1.3	1.0	ns
F	85.7	95.3	92.3	93.0	9.2	+	2.1	1.9	ns	5.2	9.0	ns
U	1.6	1.4	1.7	1.5	0.8	ns	0.1	0.2	ns	-0.2	0.8	ns
R+L	12.7	3.3	6.0	5.5	8.7	+	-2.3	1.8	ns	-5.0	8.5	ns
P												
I	12.2	12.7	13.0	13.5	0.4	ns	0.8	0.1	***	0.5	0.4	ns
F	84.1	95.6	81.1	100.7	16.8	ns	1.1	5.9	ns	15.6	15.8	ns
U	3.1	3.7	2.0	3.0	0.9	ns	-0.9	0.4	+	0.8	0.8	ns
R+L	12.8	0.7	16.9	-3.7	16.6	ns	-0.2	5.8	ns	-16.4	15.5	ns

a,b,c, see Table 6.3

Table 6.9 Daily water intake (I, kg/cow) and output (kg/cow) in urine (U) and faeces (F) during steady state periods, and the DM content (%) of faeces (FDM) for HS and LS cows offered diets of high (HR) and low (LR) K:Na ratio.

	HR		LR									
	HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
I	24.6	27.6	24.5	26.6	1.9	ns	-0.6	0.8	ns	2.6	1.7	ns
U	12.4	15.2	10.0	13.6	1.7	ns	-2.0	1.1	ns	3.2	1.3	+
F	8.6	7.0	9.1	6.3	1.7	ns	0.1	0.5	ns	-2.2	1.6	ns
FDM	12.1	14.7	12.6	16.4	2.0	ns	1.1	0.3	*	3.2	1.9	ns

a,b,c see Table 6.3

The percentage of DM in the faeces was higher for cows when offered the LR diet than when offered the HR diet (Table 6.9). There was no difference between susceptibility groups in faecal DM percent.

Analysis of the urinary and faecal output of minerals as a percent of intake during steady state periods (Table 6.10) includes an estimate of the percentage of intake which was retained and which includes that proportion lost by leakage from the fistula. The leakage of Na, as a percent of intake, in the second steady state period ranged from 0-79% for individual cows and days, averaging 30.5%. The leakage of K (including contaminated samples) ranged from 0-21% and averaged 5.5%. No analyses of the concentrations of Mg, Ca or P in rumen fluid were carried out to determine the quantities lost by leakage.

There were no differences between susceptibility groups or effects of diet on the proportion of Na, K, Mg, Ca or P intake excreted in urine or faeces, except that more P was excreted in urine of cows when offered the HR diet than when offered the LR diet, and HS cows excreted more K in faeces than did LS cows, both in quantity of K and as a percent of K intake. The increased faecal loss of K was due to an increase in concentration of K in the faeces rather than to an increase in faecal DM output.

There were no correlations between the concentration of K in plasma and either the concentration of K in the faeces or the faecal K output when the data were plotted for all cows.

Digestibility of the diets

The data for one LS cow when on the HR diet during the second steady state period were omitted from analysis of apparent digestibility of the diets because the cow had diarrhoea on 3 of the 6 days of collection. This reduced the apparent nitrogen (N) digestibility by 18.8% and digestibilities of DM, organic matter (OM) and energy (E) by 3-10% for this cow compared to the averages for the other cows. This cow's data were not excluded from the previous urine and faeces mineral analyses because her data lay within the range of values obtained for the other cows.

Apparent digestibility coefficients were significantly lower for the LR diet than for the HR diet (Table 6.11). There was no significant difference between susceptibility groups in apparent digestibility of OM or N but LS cows had significantly higher digestibility coefficients than HS cows for DM and E, for both diets.

Table 6.11 Apparent digestibilities (%) of DM, organic matter (OM), nitrogen (N) and energy (E) when HS and LS cows were offered diets of high (HR) and low (LR) K:Na ratio.

	HR		LR									
	HS	LS	HS	LS	sed[a]	sig.	LR-HR[b]	sed	sig.	LS-HS[c]	sed	sig.
DM	71.0	74.8	65.6	68.7	1.9	ns	-5.8	1.1	**	3.5	1.6	+
OM	74.9	77.6	70.6	71.0	1.7	ns	-5.4	0.8	**	1.5	1.5	ns
N	69.6	72.5	65.9	69.9	2.1	ns	-3.1	1.1	*	3.5	1.8	ns
E	69.8	72.7	65.5	69.4	1.8	ns	-3.8	0.9	*	3.4	1.5	+

a,b,c see Table 6.3

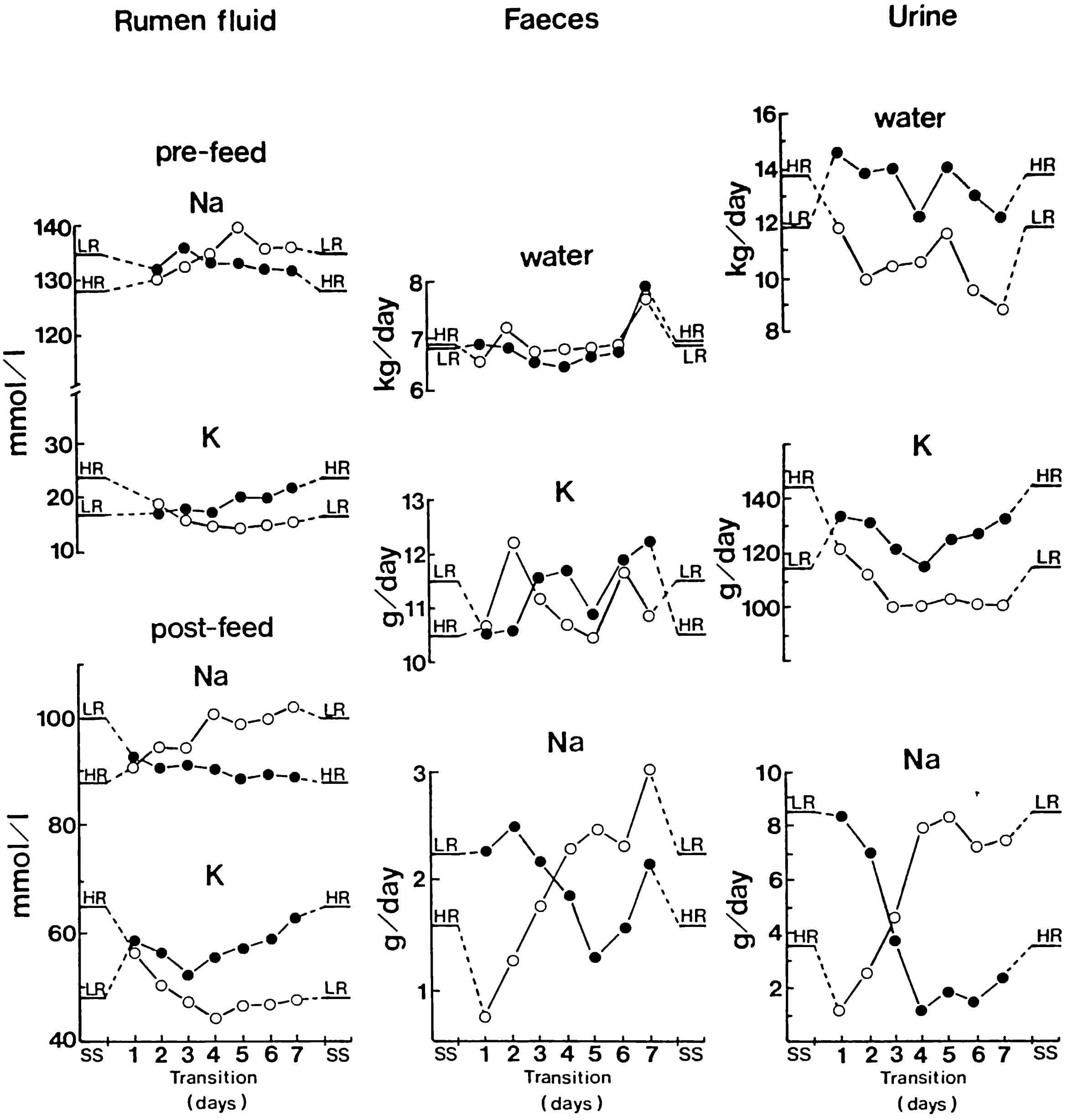


Figure 6.4 The change in concentrations of Na and K in rumen fluid before and after feeding and daily outputs of Na, K and water in faeces and urine of all cows for 7 days after a change between diets of high (HR) and low (LR) K:Na ratio. Steady state (SS) values for each diet are indicated.

6.3.7 Changes over the transition period

The changes over the first 7 days following each change in diet for some rumen fluid, urine and faeces variables are shown in Figure 6.4. Each point is the average for all cows, using data from the 2 transition periods and their preceding steady states. Blood and saliva data (not shown) showed no consistent trends over the transition periods. For all variables there were no significant differences between susceptibility groups, nor interactions between susceptibility group and diet, in the pattern of change over the 7 days of transition.

Changes in the concentrations of Na and K in rumen fluid induced by changing diet were complete within 4-7 days. The changes were possibly more rapid when changing from the HR diet to the LR diet, than from the LR to HR diet.

Changes in the outputs of water, Na and K in urine and faeces were variable, probably due in part to the day-to-day variation in leakage of rumen fluid as already noted.

6.4 DISCUSSION

The quantity of digesta in the reticulo-rumen was increased both before and after feeding in the HS and LS cows when offered the low K:Na ratio diet compared to the high K:Na ratio diet, but LS cows had less digesta before feeding than HS cows irrespective of diet. The 2.5 kg difference due to diets in the quantity of digesta before feeding was associated with a 7 mmol/ℓ increase in concentration of Na and decrease in concentration of K in rumen fluid of cows when offered the LR diet than when offered the HR diet. The difference of about 6.6 kg between susceptibility groups in the quantity of digesta was not associated with any differences in the concentrations of Na or K in rumen fluid.

The daily Na intake of 9 g/cow when offered the HR diet is in excess of the estimated requirements for non-lactating, non-pregnant cows (Towers & Smith 1983). The concentrations of Na and K of 125-130 mmol/ℓ and 22-25 mmol/ℓ, respectively, in rumen fluid samples taken before feeding indicate an adequate Na status of cows offered the HR diet (Bailey 1961b; Scott 1966; Warner & Stacy 1972a), although the concentrations of Na and K in saliva of 126-136 mmol/ℓ and 13-17 mmol/ℓ, respectively, and Na:K ratios of 10-12, indicate that Na status may have been marginally low (Bailey & Balch 1961b; Towers 1983). This was probably due to losses of Na from the fistula. The cows responded to the higher Na intake of 21 g/day when offered the LR diet by increasing the concentration and quantity of Na in the rumen fluid, and by increasing the concentration of Na and decreasing the concentration of K in saliva by 5 mmol/ℓ and slightly increasing, to about 14, the Na:K ratio. The decrease in concentration of K in the rumen fluid of cows when offered the LR diet than when offered the HR diet was not only due to dilution from the increase in the quantity of fluid, but also to a reduction in the quantity of K in the rumen fluid of cows offered the LR diet.

The effect of diet on the concentrations of Na and K in rumen fluid after feeding were about twice those observed before feeding, indicating that diet had relatively little effect on resting state concentrations. The total Na + K concentration in rumen fluid before feeding was not affected by diet. A greater shift in the resting state concentrations of Na and K in rumen fluid has been achieved in sheep by a more extreme range of intakes of Na and K (Dobson & McDonald 1963; Warner & Stacy 1972a) or under Na deficiency (Scott

1966). The concentration of K in rumen fluid after feeding was affected by diet to a greater extent than was the concentration of Na, probably because the diet is the major source of K whereas saliva is the major source of Na, even on diets relatively high in Na. The total Na + K concentration after feeding was higher after offering the HR diet than the LR diet, reflecting the increased K intake.

Concentrations of Na and K and the Na:K ratio in saliva were similar before and after feeding. There was a small increase in concentration of Na and possibly osmolality of plasma in response to feeding, although they were less than those previously reported for sheep offered chaffed hay (Stacy & Warner 1966; Carr & Titchen 1978). There was no effect of diet on concentrations of Na and K in plasma, although under more extreme Na and K intakes than used here, plasma K concentration in sheep has been increased by both high K intakes (Warner & Stacy 1972a) and high Na intakes (Hemsley *et al*. 1975).

Changing the diet involved changes in intake of both Na and K, and any effects of diet on the variables measured cannot be ascribed to either ion on the basis of the results obtained. However, changes in K intake have been shown, in sheep, to have more marked effects on Na and K metabolism than changes in Na intake, unless the animal is Na deficient. This is due to the actions of K on the rumen and kidneys. Changing from a low to a high K intake can induce a transient Na-deficient state even when Na intake is adequate (Dobson *et al*. 1966; Warner & Stacy 1972a). The increase in concentration of K in rumen fluid arising from an increased intake of K may result in an increase in absorption of both K and Na from the rumen (Dobson *et al*. 1966; Stacy & Warner 1966; Scott 1967; Warner & Stacy 1972a,b), and an increase in excretion of Na in the urine (Dobson *et al*. 1966; Dewhurst *et al*. 1968; Simpson 1969; Warner & Stacy 1972a). The effects are reversed by changing to a low K diet. A high intake of K with an adequate or high intake of Na does not affect the concentrations of Na and K in saliva even though rumen fluid concentrations change (Warner & Stacy 1972a). A high intake of K combined with a low intake of Na results in a decrease in concentration of Na and increase in concentration of K in saliva of sheep through the action of aldosterone on the parotid gland (Blair-West _et al_. 1967). Dobson (1963) noted that the aldosterone-like response of salivary Na:K ratio seen in cows changed from a low to a high K diet varied greatly in timing and intensity between animals. The comparison of the magnitude of changes in composition of rumen fluid and saliva in response to the HR and LR diets with the data obtained above on sheep, suggests that the effects of Na and K on kidney and adrenal function of the HS and LS cows would have been minimal.

The quantity of fluid in the reticulo-rumen increased both before and after feeding in cows when offered the LR diet than when offered the HR diet. The concentration and quantity of Na in digesta were higher on the LR than on the HR diet. An increased quantity of Na in digesta, resulting in an increase in fluid in the reticulo-rumen, was suggested to be the cause of an increase in live weight observed in sheep after changing from a low Na to a high Na diet (Dobson _et al_. 1966). However, Warner & Stacy (1972a) only observed this effect in sheep on very high intakes of Na of 580 mequiv/day, where a 15% increase in both volume of digesta and outflow rate from the reticulo-rumen were observed without any change in concentrations of Na and K in rumen fluid or saliva. An increase in the quantity of Na in digesta suggests either that Na was retained during the transition period between diets, or that it was redistributed between the gut and body fluids. There was no evidence of a decrease in concentration of

Na in plasma of cows when offered the LR diet than when offered the HR diet, although cows offered the LR diet had slightly greater PCV values. This indicates that a lower plasma volume may have accompanied the retention of Na in the reticulo-rumen and that both water and Na may have moved from the extracellular fluid into the reticulo-rumen in cows offered the LR diet. Alternatively, Na and water may have been retained in the reticulo-rumen through a reduction in excretion of Na in faeces or urine when changing from the HR to the LR diet. Urine volume during the 7 days following the change in diet tended to be lower than the steady state value for the LR diet (Figure 6.4), possibly indicating retention of fluid during that time. A corresponding loss of retained water when changing from the LR to the HR diet was not apparent. However, the quantities of fluid involved in the change in digesta quantity were small, and in view of the effect that leakage of rumen fluid may have had on urine and other variables, neither blood nor urine data are likely to reliably indicate the source of the increased quantity of Na and water in digesta of cows offered the LR diet.

There was no difference between cows offered the HR or LR diets in total water intake, although the cows appeared to respond to the lower water content of herbage when offered the LR diet than the HR diet by increasing the quantity of water drunk. Adding NaCl to the diet of sheep increased volume of digesta after but not before feeding (Hemsley 1975), an effect attributed to an increase in water intake. An increase in water intake has also been observed when the K content of the diet was increased (Warner & Stacy 1972a), although high K intakes did not change volume of digesta (Scott 1967; Warner & Stacy 1972a).

The increase in the quantity of fluid in the reticulo-rumen of cows when offered the LR diet than when offered the HR diet may have been due to the lower digestibility of the LR diet rather than to its higher Na concentration, but digestibility of the diet would be expected to affect the quantities of both DM and fluid in digesta. High intakes of Na by sheep, up to 3.1% of the DM content of a hay and barley diet (Moseley & Jones 1974) or 150 g NaCl/day added to 600 g concentrates (Hemsley et al. 1975) have decreased organic matter digestibility by 5 and 24%, respectively, although Cardon (1953) found no effect of salt intake on the digestibility of cellulose or energy in cows consuming lucerne hay. The difference in digestibility between the HR and LR diets may have reflected differences in species composition (Table 6.1) rather than mineral content. For example, the LR herbage in Pasture B contained a greater proportion of paspalum than the other herbages, and this may have been of lower digestibility than the ryegrass and white clover components (Coup & Dunlop 1951; E.R. Thom, unpublished data).

There was no difference between susceptibility groups in water intake, although HS cows increased their drinking water intake to a greater extent than did LS cows when offered the LR diet than when offered the HR diet. Lack of a difference between susceptibility groups in total water intake was similar to the results obtained in Experiment 6, but not to Experiments 3 and 5 where LS cows drank more than HS cows when offered pasture. In contrast, the difference between susceptibility groups of 6.6 kg in the quantity of digesta in the reticulo-rumen before feeding is intermediate between the difference of 9.2 kg observed on pasture in Experiment 3 and differences of 2.3-3.6 kg observed in Experiments 5 and 6. This, combined with the lack of an effect of diet on the total water intake despite an effect of diet on quantity of digesta, suggests that water intake *per se* is not a major determinant of the quantity of digesta or of the quantity of digesta in HS cows relative to that in LS cows.

As in previous experiments, LS cows lost less fluid from the reticulo-rumen during feeding and more fluid after feeding than did HS cows. The calculated net inflow of fluid after feeding did not differ significantly between susceptibility groups despite a 2.5% higher dilution rate in LS cows than in HS cows, due in part to the lower quantity of fluid being diluted in LS cows (56.2 vs 59.1 kg at 0 h for LS and HS cows, respectively).

The net loss of fluid during feeding was lower, and net inflow to the reticulo-rumen and outflow to the omasum after feeding higher, in cows offered the LR diet compared to the HR diet. The mechanism of the difference in loss during feeding is uncertain. It is unlikely to be the result of inhibition of salivary flow in cows offered the HR diet due to increased osmolality of rumen fluid or plasma (Warner & Stacy 1977; Carr & Titchen 1978), since there was little effect of diet on osmolality of rumen fluid. Possibly it resulted from a difference in physical characteristics between diets; saliva production may increase on fibrous diets (Bailey 1961b) or in response to increased DM content of the diet (Meyer *et al*. 1964). The differences between the diets in fibre and DM contents were small, but so was the effect of diet on loss during feeding.

Excretion of Na in faeces and urine averaged 14% and 40% of the Na intake, respectively, and of K, 7% and 80% of the intake, respectively. Corresponding values for cows offered pasture in Experiment 1 were 43% and 39% for Na and 11% and 82% for K, indicating that faecal loss, particularly for Na, may have been affected to a greater extent than urinary loss by leakage of rumen fluid from the fistula. In Experiments 1, 2 and 8, the output of K in faeces was higher for HS than for LS cows on a range of diets. Three of the 12 cows (2 HS, 1 LS) on which mineral measurements were made in Experiments 1 and 2 were also used in Experiment 8. That the faecal K data from this experiment are in agreement with the earlier experiments with non-fistulated cows suggests that the effect of leakage from the fistula on K output in faeces was minimal.

There was a non-significant difference between susceptibility groups in the proportion of the intake of K which was excreted in the urine, of similar magnitude to the difference in faecal K excretion but with LS cows excreting more than HS cows. Urine data were more variable than faeces data, as indicated by the higher standard errors of the differences between means, therefore although the difference between susceptibility groups in K output in urine was not significant, the total output in urine and faeces by HS and LS cows was similar. This suggested that there were no differences between HS and LS cows in net retention of K, rather than that the difference in faecal output was associated with a difference in distribution of K between the gut and body fluids.

The higher excretion of K in faeces by HS cows was possibly associated with their lower concentration of K in plasma, compared to LS cows, although there was no correlation between K concentration or output of K in faeces and concentration of K in plasma.

Leakage of fluid and electrolytes from the fistula was a problem in this study. Part of the problem specifically arose in the digestibility stalls when the cannulae of cows lying down were caught on the sides of the stalls, particularly for the larger LS cows. The problem worsened as the experiment progressed because the cows spent more time lying down. There was a range between cows of those that never leaked (2 cows), those that leaked on some days and in variable amounts (4 cows) and those that consistently leaked (2 cows). HS and LS cows were equally represented in each of these groups.

Diet had little effect on the composition of body fluids in this experiment despite a 2-fold range in Na intake and shift in K:Na ratio. The average ratios of 19 and 7 obtained on HR and LR diets, respectively, can be compared to those of Turner (1981) who found that pastures associated with a high incidence of bloat had 3.13% K and 0.09% Na (ratio 35) and those with a low incidence of bloat had 2.70% K and 0.18% Na (ratio 15). The concentrations of K in both the HR and LR diets were higher than those found by Turner, and the concentration of Na in the HR diet was higher than that of Turner's low ratio pasture. If the absolute concentration of K or Na in the diet is important in its effects on bloat, rather than the ratio, then both the HR and LR diets may have been too high in K (and therefore be capable of causing bloat) or too high in Na (and therefore be associated with a low incidence of bloat). If the value of the ratio is important, the ratios of both the HR and LR diets may have been within the range of diets associated with a low incidence of bloat. On the basis of the results obtained here, it appears that the mechanism of any effects of a low K, high Na diet on bloat is not associated with that which affects the quantity of digesta in the reticulo-rumen and which may affect an animal's susceptibility to bloat.

CHAPTER 7

GENERAL DISCUSSION

Summary of observations

The aim of this thesis was to investigate the digestive characteristics of two groups of cattle differing in susceptibility to bloat and identify any differences which might contribute to this variation in susceptibility or resistance. To refresh the reader's mind the experiments are summarised by their short titles (Table 7.1), their main results (Table 7.2), and by a more detailed summary of the quantities of digesta in the reticulo-rumen of HS and LS cows (Table 7.3).

The difference between susceptibility groups in the quantity of digesta in the reticulo-rumen after 21 h (non-lactating cows) or 18 h (lactating cows) of fasting was statistically significant only when the cows were offered pasture in Experiment 3 and when offered 2 pasture diets in Experiment 8. In all experiments, however, including that with lactating cows where the quantity of digesta in the reticulo-rumen was greater for LS than for HS cows, the weight of digesta as a percent of the estimated reticulo-rumen capacity was lower for LS cows than for HS cows (Table 7.3). Digesta fill was 7 to 45% greater for HS than for LS cows, despite both susceptibility groups consuming per unit live weight similar quantities of DM and, in most experiments, water.

The greatest difference between susceptibility groups in the quantity of digesta occurred when the quantities averaged about 33 kg and 40 kg for LS and HS cows, respectively. The difference was least when both susceptibility groups had more than 37 kg digesta in the reticulo-rumen. These observations suggest that LS cows decreased

Table 7.1 A summary of the experiments

Experiment	Short title
1	Intake and digestibility study with non-lactating cows offered hay and pasture
2	Intake, digestibility and milk production study with cows offered pasture and pasture plus hay.
3	Restricted feeding of hay and pasture to non-lactating cows.
4	*Ad libitum* feeding of pasture to lactating cows.
5	Manipulation of the quantity of digesta in the reticulo-rumen after feeding.
6	Restricted feeding of pastures of low, medium and high maturity.
7	Intraruminal addition of water and electrolyte solutions.
8	Restricted feeding of diets of high and low K:Na ratio.

Table 7.2 A summary of results indicating whether values for LS cows were greater than (+), similar to (=) or less than (-) those for HS cows.

Parameter		Diet	Experiment	Result
DM intake (*ad libitum*)		hay, pasture, pasture + hay	1,2,4	=
Water intake		hay, pasture	1,2,3,6,8	=
		pasture, pasture + hay	2,3,5	+
Yields of	milk	pasture, pasture + hay	2	+
	fat, protein, lactose	pasture, pasture + hay	2	=
Digestibility		hay, pasture, pasture + hay	1,2	=
		pasture	8	+
Digesta quantity		hay, pasture	3,4,5,6	=
		pasture	3,8	-
Digesta DM %		hay, pasture	3	=
		pasture	4,5,6,8	+
Rumen fluid				
	osmol, Na, K	hay, pasture	3,4,8	=
	dilution rate	hay, pasture	3,4	=
		pasture	8	+
Faecal K	output	hay, pasture, pasture + hay	1,2,8	-
Saliva	osmol, Na	pasture	4,8	=
	K	pasture	4	-
		pasture	8	=
	protein	pasture	4	-
	CO_2	pasture	4	=
Blood	PCV	pasture	4,8	=
Plasma	osmol, Na	pasture	4,8	=
	K	pasture	4	=
		pasture	8	+

Table 7.3 A summary of the quantity of digesta[a] (kg) in the reticulo-rumen and digesta quantity as a percent of reticulo-rumen capacity[b] of HS and LS cows.

			Quantity		% capacity	
			HS	LS	HS	LS
Non-lactating, pregnant						
Expt 3	Hay		44	42	48	44
	Pasture		41	32	48	33
Non-lactating, non-pregnant						
Expt 5	Pasture		41	37	43	37
Expt 6	Pasture	L[c]	36	34	38	34
		M	43	39	45	39
		H	46	46	48	45
Expt 8	Pasture	HR[d]	38	32	40	32
		LR	41	34	43	34
Lactating						
Expt 4	Pasture		38	40	35	31

[a] 21 or 21.5 h after feeding for non-lactating cows, 18 h after feeding for lactating cows

[b] Capacity measured in Experiments 3, 4, 5; capacity in Experiments 6 and 8 assumed to be that in Experiment 5

[c] Herbage of low (L), medium (M), and high (H) maturity

[d] Herbage of high (HR) and low (LR) K:Na ratio

their quantity and fill of digesta relative to that of HS cows under some experimental, physiological or dietary conditions. The exception to this occurred on the pasture of lowest maturity in Experiment 6, when the quantity of digesta in HS cows was lower than that found in the same cows in other experiments. The low maturity diet also resulted in foamy digesta in 4 HS and 3 LS cows, as did the 2 diets in Experiment 8, although no bloat occurred in either experiment. If it is assumed that the quantity of digesta (or fill) is positively related to the occurrence of bloat (this was not re-tested in this thesis) then either: (1) LS cows could express their low susceptibility on bloat potent diets by decreasing their quantity of digesta relative to that of HS cows, or (2) HS cows may bloat when they fail to respond to factors which facilitate the loss of digesta from the reticulo-rumen.

Since LS cows sometimes bloat, and HS cows vary in their occurrence and degree of bloat, it is possible that the quantity of digesta in the reticulo-rumen is one of several factors either causing bloat or, if not causing it, affecting its severity. Pasture of high digestibility, such as that which might cause bloat, might be expected to result in less digesta in the reticulo-rumen relative to that on more mature diets (for example the comparison of the pastures differing in maturity in Experiment 6). It could be speculated that HS cows may have bloated had their quantity of digesta when offered the low maturity diet not been less than when offered the more mature diets in Experiment 6. Alternatively, although HS cows had more digesta of a foamy nature than LS cows when offered pastures in Experiment 8, some other factor(s) necessary for bloat was not present.

Factors contributing to the variability in results between the experiments

Despite the abundance of literature on the effects on the quantity of digesta in the reticulo-rumen of factors such as the level of intake, diet, physiological state, mineral supplementation, weather and various hormones, there has been little attempt to explain the causes of the variation between animals that has occurred. The control of the quantity of digesta is complex; a change in one factor resulting in a range of responses, a possible net effect being a change in the quantity of digesta in the reticulo-rumen. This study, which involved the investigation of a limited number of factors, was hindered by a variability between experiments that could not be associated directly with the experimental factor being investigated. The diet was considered to be the most probable cause of this variability, since there is limited control of dietary composition when pasture is the sole diet. The effects of specific dietary constituents on digesta parameters appears to be the least well researched aspect involving quantity of digesta, but may have been the most important factor affecting this study.

There were others factors additional to diet that differed between experiments and that need to be considered when evaluating the results of the various experiments. These included the level of DM intake and the feeding regime, water intake, physiological state and Na status of the cows.

1. Diet

The extent that dietary factors affected the variation between experiments in the quantity of digesta in HS cows compared to that in LS cows cannot be assessed from these results. Even so it appeared that a difference between susceptibility groups was least likely on mature pasture or hay diets. Experimental investigation of the plant constituents possibly contributing to differences in digesta is difficult, as the concentrations of many constituents change with changes in plant maturity. The occurrence of a difference between susceptibility groups in the quantity of digesta when the cows were offered pasture that had been frozen and thawed (Experiment 3) suggested that freezing and thawing did not destroy, and possibly enhanced, the effects of the dietary constituents or characteristics involved. Freezing and thawing of pasture has been shown to decrease the contents of soluble nitrogen, soluble protein and ash, and increase the content of water soluble sugar (Bryant & Newth 1975).

With the exception of Experiment 8, no information was obtained on the species composition of the diets consumed. Pure swards of ryegrass or clover may have been preferable to mixed pastures, but were not available. The 3 pastures offered in Experiment 6 (low, medium, high maturity) were harvested from the area referred to as Paddock A in Experiment 8. However, whereas LS cows had less digesta in the reticulo-rumen than HS cows in Experiment 8, the susceptibility groups did not differ in the quantity of digesta in Experiment 6. The pastures were harvested in spring and autumn for Experiments 6 and 8, respectively, and may have differed in species composition since the white clover content of pastures containing ryegrasses and white clover and grazed by dairy cows is higher in autumn than in spring (Hutton *et al*. 1967).

An increase in the quantity of digesta in the reticulo-rumen was associated with 2 factors, an increase in maturity of the pasture and a change from a high to a low K:Na ratio. The K:Na ratios of the 3 pastures differing in maturity in Experiment 6 ranged from 38 to 47, considerably higher than the ratios of 26 or less in Experiments 3, 4, 5 and 8. The higher ratios in Experiment 6 arose from low concentrations of Na (0.06-0.09%) rather than high concentrations of K. It appears, therefore, that changes in the concentration of Na or in the K:Na ratio of the diet were not responsible for the observation in Experiment 6 that the quantity of digesta increased with increasing pasture maturity. The decline in K concentration with increasing maturity that was observed in Experiment 6 has been noted previously (Fleming & Murphy 1968; Whitehead & Jones 1969).

While the decrease in Na intake in Experiment 3 may have contributed to the lower quantities of digesta when the cows were offered pasture rather than hay, it appears unlikely from the data obtained in Experiment 8 that the changes in Na and K intake in Experiment 3 contributed to the observed difference between susceptibility groups in quantity of digesta when the cows were offered pasture.

Apparent digestibilities of DM and energy in both diets in Experiment 8 were higher for LS cows than for HS cows. This contrasts with Experiments 1 and 2 where there were no differences between susceptibility groups in digestibility coefficients. The differences in Experiment 8 were associated with a difference between susceptibility groups in the quantity of fluid in the reticulo-rumen but not of DM in the reticulo-rumen or loss of DM during or after feeding. The possibility arises that apparent digestibility coefficients may differ for HS and LS cows when the diet results in a difference in the quantity of digesta in the reticulo-rumen. The digestibility coefficients in Experiments 1 and 2 may have been obtained when there were no differences in the quantity of digesta in the reticulo-rumen. Neither DM loss from nylon bags nor quantity of digesta differed between susceptibility groups in Experiment 4.

2. Level of DM intake

The available evidence (Experiments 1, 2, 4) indicated that *ad libitum* intakes of HS and LS cows were in proportion to live weight, whether or not the cows were fistulated. Offering food in proportion to live weight in subsequent experiments was aimed at avoiding any effects on digesta turnover of a difference in intake relative to live weight or, more appropriately, relative to reticulo-rumen capacity since this was proportional to live weight. If the live weights were measured when circumstances allowed greater quantities of digesta in HS than in LS cows, the amount of food offered to HS cows would have been slightly overestimated compared to that for LS cows.

The maximum difference between susceptibility groups in the quantity of digesta in the reticulo-rumen occurred when DM intakes were the lowest, about 3.2 kg pasture DM in Experiment 3, and when intakes expressed on a live weight basis were slightly but not significantly lower for LS cows than for HS cows. However, the quantity of digesta also differed between susceptibility groups in Experiment 8 when DM intakes were 4.3-4.5 kg. In addition, there was no indication that the addition or subtraction of digesta in Experiment 5, equivalent to intakes of 3.4-6.0 kg DM, affected the quantity of digesta in HS cows differently from that in LS cows. An assumption made in Experiment 5 is that the effects of a net gain or loss in DM and fluid after addition or subtraction of digesta were similar had those amounts been consumed, and that short-term changes in quantity of digesta after feeding had similar effects on subsequent loss and quantity of digesta as did long-term adaptation to a low level of intake.

The lack of a difference between susceptibility groups in the quantity of digesta in the reticulo-rumen when offered pasture *ad libitum* (Experiment 4) might indicate that HS and LS cows do not differ in quantity of digesta when intake is not restricted. Alternatively, factors other than the level of intake may have resulted in the lack of a difference in the quantity of digesta in Experiment 4, as occurred when the cows were offered hay in Experiment 3 or pasture in Experiments 5 and 6. The possibility exists that the *ad libitum* intakes in Experiments 1 and 2 were measured on hay and pasture diets which did not result in a difference between susceptibility groups in the quantity of digesta, as occurred in Experiment 4. HS and LS cows may differ in their *ad libitum* intakes when offered diets that result in the two susceptibility groups having different quantities of digesta in the reticulo-rumen.

3. Water intake and the effect of withholding drinking water

LS cows consumed more water than did HS cows when offered pasture in Experiment 3, raising the possibility that the higher water intake of LS cows was associated either directly, or via the concentration of some rumen constituents, with their lower quantity of digesta. This seems unlikely since when the results from all experiments are considered, differences between susceptibility groups in water intake and digesta quantity could occur independently of each other. Other available data also indicate variable or poor correlations between water intake and rumen fluid volume. Willes *et al*. (1970) found that sheep with the lowest water intake had the lowest volume of fluid in the reticulo-rumen, but Hartnell & Satter (1979) found no relationship between these variables; 2 lactating cows with the same water intake had a difference of 30 kg in weight of fluid in the reticulo-rumen.

The effect of withholding water on measurement days on the subsequent quantity of digesta at 21 h appeared to be minimal. This is despite the possibility that it affected the rates at which plasma volume expanded and rumen osmolality decreased following feeding. Simpson (1969) observed that less water was drunk by sheep if drinking water was withheld for several hours after feeding on hay, suggesting that thirst was partially satisfied by internal redistribution of fluid. This fluid could be drawn from the gut, potentially affecting the quantity of digesta in the reticulo-rumen. In the present experiments, in situations where the water intakes prior to measurement of the quantity of digesta were higher for LS than for HS cows, more fluid may have been drawn from the gut of LS cows than HS cows to satisfy thirst when water was withheld.

There is uncertainty as to why water intake differed in some experiments. Intake of water during and after feeding occurs in response to the increase in osmotic pressure of rumen fluid and plasma (Warner & Stacy 1965; Ternouth 1967; Carr & Titchen 1978) and to the decrease in plasma and extracellular fluid volume (Blair-West & Brook 1969; Christopherson & Webster 1972) that occur during consumption of food. The effects of feeding on plasma are exaggerated by feeding once daily, compared to more frequent feeding (Dooley & Williams 1975). The osmolality of rumen fluid from lactating cows fed twice daily in Experiment 4 was never found to be hypotonic to blood, and this effect more closely resembled those found in animals fed hourly rather than once daily (Dooley & Williams 1975). In the present experiments the time of access to water was restricted, drinking was rapid, and intake was probably completed before the water was absorbed and therefore while plasma osmolality was still elevated. This implies that some kind of pharyngeal or gastrointestinal metering of water intake may have been involved. The water drunk may not all mix with digesta as Woodford et al. (1984) found that 5-18% of the water consumed after feeding, bypassed the reticulo-rumen. The differences between susceptibility groups in water intake on some diets may have reflected individual differences in osmolality and volume changes of plasma, possibly resulting from differences in the rate of eating. The rate of eating is known to affect the extent to which rumen fluid volume increases during feeding, with rate of fluid inflow far exceeding rate of fluid outflow in rapid eaters (Warner & Stacy 1968b). Variations in the rate of secretion of saliva and other fluids in the gut and in reticulo-rumen motility may also have contributed to the differences in water intake; the former because of their effects on plasma volume and tonicity and the latter because of its possible importance to transport and absorption of water and electrolytes. The extent that these factors contributed to the differences in water intake cannot be assessed from the data.

Feeding is often preceded by a diuresis which varies in magnitude between animals (Simpson 1969), and the magnitude may possibly affect subsequent drinking behaviour. Increased water intake by sheep in response to drug-induced diuresis has been found to be rapid and to quantitatively replace the water lost (Zimmerman et al. 1978). Stress has also been associated with diuresis (Anderson 1961). The cows may have been stressed due to non-routine procedures and an unfamiliar environment, particularly in Experiment 3 which was the first experiment involving the fistulated cows.

Loss of rumen fluid and Na by leakage from the fistula may also have affected water intake. Abraham et al. (1976) found that water intake was significantly increased in sheep depleted of water and Na by loss of parotid saliva, although the increase did not equal the water deficit. Higher water intakes by LS cows might imply greater loss of rumen fluid by LS cows than by HS cows. LS cows lost slightly more fluid from the fistula than HS cows in Experiment 8, but there was no difference between susceptibility groups in water intake in that experiment, suggesting either that a very large difference in leakage loss would have been required to affect water intake, or that leakage and intake were not related.

4. Physiological state

There was no evidence that reticulo-rumen capacity decreased with advance in pregnancy and change of diet from hay to pasture in Experiment 3. Pregnancy was found by Weston et al. (1983) to be associated with a decrease in the quantity of DM and total digesta in the reticulo-rumen, when intake was held constant. They assumed that changes in endocrine status or physical effects of the foetus were involved. Their data were obtained at 3-15 weeks pre-partum, similar to those in Experiment 3 which was 8-15 weeks pre-partum. Data from Experiments 5 and 8 indicated that differences between susceptibility groups in water intake and in the quantity of digesta in the reticulo-rumen could occur in non-pregnant animals. However, cows in Experiment 8 received progesterone to control oestrus. The circulating level of progesterone after insertion of the controlled release (CIDR) devices is maximal 1-2 days after insertion; it then declines over the following 10-12 days. The blood progesterone levels after insertion of devices similar to the CIDR (Roche et al. 1981) are within the range of those found during the latter half of pregnancy (Donaldson et al. 1970; Robertson 1972). The hormonal status of cows in Experiment 8 may have been, therefore, similar to that in Experiment 3. Progesterone competitively blocks the action of aldosterone and causes excretion of Na in the urine, but in the absence of aldosterone has no effect on renal excretion of Na (Landau 1973). Individual cows are likely to have responded differently to the dose of progesterone administered in the CIDR, and any effect on renal excretion will have depended on whether cows were induced into an aldosterone response by Na deficiency (see below).

The alternative to administration of progesterone in Experiment 8, no control of oestrus, would have resulted in marked changes in DM intake, water intake, excretion of Na and K in urine and faeces, and in Na and K composition of body fluids during and for some days after oestrus (Michell 1979).

Other aspects of physiological state, namely fatness and lactation, affect reticulo-rumen capacity. Fatness may physically limit capacity (Tayler 1959). When the live weights of the 4 fat cows in Experiment 3 were reduced by 25 kg for each condition score (Grainger et al. 1978; Macmillan & Bryant 1980) above the average score of the thin cows, the capacity of the fat cows was 19% of live weight, still lower than the 25% obtained for the thin cows. The fat cows were those used by Cockrem et al. (1983). If fatness decreases the distension of the reticulo-rumen, then severity of bloat, in terms of distress of the animal, may have been enhanced during Cockrem's exchange experiments.

Lactation resulted in a reticulo-rumen capacity that was 34% greater than in non-lactating cows, an increase similar to previous estimates using the same technique (Tulloh & Hughes 1965). The largest percentage increases occurred in cows that lost the most condition between Experiments 3 and 4.

The technique used to estimate capacity of the reticulo-rumen probably overestimates capacity for food since it makes no allowance for gas space and the specific gravity of digesta is less than 1.0. Using the technique for comparative estimates assumes that the degree of distension of the reticulo-rumen in response to filling with water is similar for both susceptibility groups.

5. **Na status**

The cows were in a marginal to low Na status in Experiment 3, and possibly marginal status in Experiment 8. The concentrations of Na and K of about 100 mmol/ℓ and 40 mmol/ℓ, respectively, in the saliva of lactating cows in Experiment 4 also indicate a marginal Na status despite supplementation of the diet with NaCl. It is possible, therefore, that the cows were in a marginal or low Na status in Experiments 5, 6 and 7, this being a condition either peculiar to these cows or specifically associated with fistulation. In sheep, there is a dose-response relationship between the circulating aldosterone level and the parotid salivary Na:K ratio (Blair-West *et al*. 1963). Based on this relationship, the salivary ratios obtained in the present experiments would indicate an increased secretion rate of aldosterone, although the relationship may differ between sheep and cattle. However, the samples of saliva obtained in the present experiments may have contained saliva from glands other than the parotid, and may have been contaminated with food or rumen fluid if the cows were eating or ruminating prior to sampling. These contaminations were avoided where possible, but the assessment of Na status using these samples is less accurate than that from parotid saliva obtained by cannulation.

The preceding discussion of factors which may have affected the variation between experiments has considered each factor in isolation, without considering that they may act in an additive or synergistic fashion. The possibility that different mechanisms affected relative quantities of digesta in different experiments cannot be excluded.

Possible mechanisms contributing to the observed differences between susceptibility groups

1. Fluid and DM loss from the reticulo-rumen

The pattern of net loss of fluid from the reticulo-rumen over 24 h differed between HS and LS cows, except in Experiment 4 when the cows were lactating. The net loss of fluid during feeding was lower and that after feeding higher, for LS cows compared to HS cows, and in Experiments 3 and 6, LS cows also lost more DM after feeding than HS cows. This difference in pattern of loss occurred irrespective of whether there was a difference between susceptibility groups in the quantity of digesta in the reticulo-rumen before feeding, suggesting that the pattern did not cause or contribute to the differences between susceptibility groups in the quantity of digesta in the reticulo-rumen.

Differences between susceptibility groups with respect to net fluid loss could have arisen from differences in salivary inflow, outflow to the omasum, or in net water movement across the rumen wall. Differences in DM loss may have arisen from differences in the extent of DM digestion and outflow of DM to the omasum.

Salivary flow and fluid dilution rate HS cows secreted slightly more saliva when collected at the cardia than did LS cows in Experiment 4. However, no differences between susceptibility groups in loss of fluid or DM during feeding were observed in this experiment and the total quantity of saliva produced over the whole feeding period was not measured. A higher production of saliva during feeding by LS cows compared to HS cows would be required to account for the observed difference in net loss of fluid during feeding. The inflow of saliva during feeding may have been higher for LS than for HS cows due to their slightly higher absolute intake of DM. Other estimates of the saliva production of HS and LS cows, indicating that LS cows produce more saliva than HS cows (McIntosh & Cockrem 1977), have been made during non-feeding periods or when saliva production was stimulated by acetic acid on the tongue. Mendel & Boda (1961) found that LS cows secreted 5% and 28% more saliva during feeding and resting, respectively, than HS cows.

In addition to a lower net loss of fluid from the reticulo-rumen during feeding for LS cows than for HS cows, losses also decreased as the maturity of the diet increased (Experiment 6) and were lower in cows offered the low K:Na ratio (LR) diet than the high ratio (HR) diet (Experiment 8). Whether one mechanism, such as an increase in saliva production, was common to these 3 situations cannot be assessed from the data. The susceptibility effect occurred over and above the effect of diet. Both the LR and mature diets were lower in apparent or _in vitro_ digestibility and K concentration and higher in DM content than the HR and less mature diets.

There were no differences between HS and LS cows in net fluid inflow to the reticulo-rumen during the 8 h period after feeding, although the dilution rate was higher for LS cows than HS cows in Experiment 8. This suggests that if susceptibility groups differed in salivary input, it was offset by a difference in movement of water across the rumen wall. Water moves in response to osmotic gradients, but only small net movements of water have been found to occur in the range of osmolality of rumen fluid that were observed in these experiments (Stacy & Warner 1966; Warner & Stacy 1968b; Willes _et al._ 1970). It is possible, however, that the rumen wall of HS and LS cows may differ in permeability to water.

The lack of a difference between susceptibility groups in the fluid dilution rate in most experiments suggests that LS cows were not diluting to below a critical level some component which causes bloat. The exception to this would be if HS and LS cows did differ in the origin of the fluid entering the reticulo-rumen. If this were so, dilution by saliva and water may have a different effect on the composition of rumen fluid.

Outflow of digesta The higher net outflow after feeding that was observed in LS cows compared to HS cows may remove digesta at a faster rate, although the difference between susceptibility groups in dilution rate or in rate of outflow were small compared to those which have been effective in reducing ruminal degradation of protein (Hemsley et al. 1975), or have been associated with a change in fermentation pattern (Hodgson & Thomas 1975; Harrison et al. 1975; Thomson et al. 1978).

Substantial losses of DM from the reticulo-rumen occur during or within several hours of feeding for animals fed once daily (Reid et al. 1979; Moseley & Jones 1984). Chewing activity is maximal 4-8 h after feeding (Waghorn & Reid 1983). Increases in outflow of digesta from the reticulo-rumen in response to an increase in intake have been found to occur during 0-3 h, rather than 3-24 h, after feeding (Moseley & Jones 1984). In lactating cows in the present experiments the rates of change of weight of fluid and DM during the 5 h feeding period were 10 and 6 times greater, respectively, than during the subsequent 18 h. A more detailed investigation of the rate of change of quantity of both fluid and DM in the reticulo-rumen after feeding may identify critical periods of differing rates of loss for HS and LS cows, as data from Experiments 3, 5 and 8 indicated that differing rates of loss occurred during the first 7-8 h after feeding, rather than subsequently. In Experiments 3 and 8 this resulted in a difference between susceptibility groups in the quantity of digesta within 7 to 8 h of feeding, and suggests that HS and LS cows may differ in the quantity of digesta before feeding when offered food at intervals of less than 24 h.

In all experiments, although only significant at some sample times in Experiments 4, 5, 6 and 8, LS cows maintained slightly higher (0.3-1.5%) DM contents of digesta, irrespective of whether there was a difference in water intake between susceptibility groups. Phillips (1961) and Thornton & Yates (1968) found that the percentage of DM in digesta was constant despite large differences in the ratio of water intake to DM intake, increasing by only 1-2% when water intake was restricted to 50% of the unrestricted level. High water intakes or the addition of rumen fluid to digesta increases the outflow from the reticulum and omasum (Ash 1962; Rumsey _et al_. 1979), but this may be due to distension of the reticulo-rumen rather than to an effect on outflow of the DM content of digesta. Data obtained in Experiment 6 suggested that the quantity of fluid lost from the reticulo-rumen changed according to intake, independently of DM loss.

Motility of the reticulo-rumen Outflow of digesta to the omasum occurs during the A sequence of contraction (Bost 1970). The outflow in one susceptibility group relative to the other may be modified by a change in the frequency of contraction, the quantity transferred per contraction, the composition of the outflow, or through outflow also occurring during the B sequence. The frequency of contraction increases in response to feeding (Reid 1963; Waghorn & Reid 1977, 1983; Colvin _et al_. 1978), and the amount transferred per contraction increases with increasing intake (Freer _et al_. 1962; Ulyatt _et al_. 1984). Freer _et al_. (1962) found that changes in the amount transferred per contraction were more important in changing outflow than were changes in the frequency of contraction. In studies on HS and LS cows, Waghorn & Reid (1984) found that the frequencies of both

A and B sequences before feeding were higher in LS cows than in HS cows. A difference such as this may have contributed to the higher outflow of digesta observed after feeding in LS cows than HS cows in the present experiments. In contrast Waghorn & Reid (1984) found that the frequency of the A sequence contraction was similar for both susceptibility groups during eating, while the B sequence frequency remained higher for LS cows. This does not readily explain the higher outflow observed in HS cows than in LS cows during feeding in the present experiments. However, differences between susceptibility groups in motility need not contribute to differences in outflow both during and after feeding. Waghorn & Reid (1984) also observed in one of two experiments that the cranial pillar descended to a lower level in LS cows than in HS cows after B sequence contractions. As the sorting and stratification of particles may influence the transfer of digesta from the reticulo-rumen, a difference between susceptibility groups such as this may change the composition of the outflow through an effect on the movement of fluid and small particles to the omasum.

The effect of the quantity of digesta in the reticulo-rumen on motility or anatomical characteristics has not been investigated in HS and LS cows. Waghorn & Reid (1984) found the cardia to be 22 cm ventral to the roof of the dorsal rumen in both HS and LS cows, but it was not clear whether their cows differed in total reticulo-rumen capacity as was observed in the present experiments. The degree of cardia exposure is important for eructation efficiency; sheep positioned so that the cardia is more deeply submerged eructate with more difficulty (Weiss 1953b). Sissons et al. (1984) found no relationship between the frequency of contraction of the reticulum and the volume of rumen fluid in steers offered several diets. Further work is required to establish whether motility has a role in affecting either the rates of loss of fluid during and after feeding or the quantity of digesta in HS and LS cows.

The concentration of Na in the rumen fluid The higher water intake in Experiment 3 of LS cows, compared to HS cows, was associated with a lower concentration of Na and osmolality of rumen fluid after feeding. Osmolality of rumen fluid before feeding did not differ between susceptibility groups. The osmolalities of rumen fluid at 7-8 h after feeding, when differences between susceptibility groups in the quantity of digesta were already apparent (Experiments 3, 8) were similar to values before feeding, but the quantity of rumen fluid continued to decrease over 7-21 h with little change in osmolality.

While it appeared that the higher water intake by LS cows than HS cows in Experiment 3 decreased the concentration of Na in rumen fluid by dilution, the concentration of K was not affected by water intake. This may have arisen from the release of K from the food, or from the difference between Na and K in absorption from the rumen. K is absorbed in response to the concentration gradient between rumen and blood (Parthasarathy & Phillipson 1953; Scott 1967; Warner & Stacy 1972b) whereas Na is actively absorbed against a concentration gradient (Warner & Stacy 1972b). Dilution of K with water may have offset absorption that would otherwise have occurred. There was, however, no effect of dilution even at the first sample time, implying a very rapid adjustment of rumen K concentration to the extra water consumed. This in turn implies rapid mixing of the water with digesta. Both of these appear unlikely since no more than 30 minutes elapsed between the end of drinking and the removal of digesta. The tendency for a positive relationship between water intake and K concentration 21 h after feeding on pasture in Experiment 3 may have been due to the inverse relationship that exists between the concentrations of Na and K in rumen fluid (Warner & Stacy 1965), thus a Na concentration lowered by dilution would be associated with a higher K concentration.

The data obtained in Experiments 7 and 8 suggested that the higher outflow of digesta from the reticulo-rumen of LS cows compared to HS cows did not occur in response to the concentration of Na in the rumen fluid.

2. Mineral metabolism

HS cows excreted a greater proportion of their K intake in faeces, and possibly less in urine, than did LS cows, and this may have been associated with a lower concentration of K in plasma of HS cows. The physiological significance of these results, particularly with respect to bloat, is unknown.

Faecal K output K is absorbed from digesta in the reticulo-rumen, omasum and abomasum, and small and large intestines (Grovum & Hecker 1973; Grace et al. 1974; Sklan & Hurwitz 1985). A difference between HS and LS cows in faecal K excretion could arise at any part of the digestive tract, and could be modified by the diet and the level of intake (Goodall & Kay 1965b; Grace et al. 1974). The pattern of change in concentrations of Na and K in the large intestine has been found to vary between individual animals, with at least 5 patterns of Na and K concentrations observed in sheep offered chaffed lucerne (Hecker & Grovum 1971; Grovum & Hecker 1973). Goodall & Kay (1965a) found differences between 2 sheep in faecal K content despite similar K concentrations in ileal digesta. Whereas absorption of Na from the gut is active, and water absorption is linked to that of Na (Grovum & Hecker 1973), absorption of K is passive and the concentration of K in faeces may be determined by the efficiency with which K is absorbed (Smith 1969). Na and K exchange in the gut may be controlled by hormones of the adrenal cortex (Smith 1969).

Plasma K concentration The difference between susceptibility groups in plasma K concentration observed in Experiment 8 was not apparent in Experiment 4 when the cows were lactating, except after 20 minutes of feeding when the HS cows showed a slightly lower concentration of K than did the LS cows. The lower concentration of K in plasma of HS cows, compared to LS cows, in Experiment 8 may have been associated with their lower (though not significant) urinary K output, as excretion of K in the urine has been found to parallel changes in plasma K concentration induced by intraruminal or intravenous administration of K (Sellers et al. 1951; Simpson 1969; Beal et al. 1973). The concentration of K in plasma could increase as a result of the accumulation of K of either gut or cell origin. Temporary increases in plasma K concentration occur when net excretion fails to match intake, such as following oestrus (Michell 1979).

There was a suggestion in Experiment 8 that the difference between HS and LS cows in the concentration of K in plasma was greater on the low K:Na ratio diet than on the high ratio diet. The concentration of K in plasma has been increased by several dietary factors, including intraruminal administration of both NaCl and KCl (Sellers et al. 1951; Hemsley et al. 1975) and saline drinking water (Tomas et al. 1973), and decreased by intravenous infusions of sucrose and urea (Warner & Stacy 1977).

The concentration of K in plasma also increases during insufflation of the rumen (Beckett et al. 1967) but it is unlikely that rumen pressure contributed to differences between susceptibility groups in plasma K both before and after feeding.

There is little evidence for genetic or breed differences in mineral metabolism. Wiener et al. (1980) reported from a study of Ayrshire cattle that the heritability of plasma K concentration was zero, although Rowlands et al. (1974) estimated a heritability of 0.40 in Hereford x Friesian calves from 12 sires. No differences between 2 breeds of sheep were found in the relative importance of urinary and faecal routes of excretion (Beal & Budtz-Olsen 1968). Jersey and Friesian cows differ in their ability to handle heat stress (Seath & Miller 1947) and Holstein cows have been found to decrease their serum and urinary K concentrations under heat stress (El-Nouty et al. 1980). It is unlikely, however, that cows were heat stressed during Experiment 8.

Small increases of 0.5-1.5 mmol/ℓ in plasma K concentration can act directly on the adrenal gland to increase the secretion rate of aldosterone (Funder et al. 1969), but these are generally responses to short-term changes in plasma K. Higher, by 0.3-0.4 mmol/ℓ, but not abnormal K concentrations have been associated with a stronger preference for Na, although not necessarily to an increased physiological need for Na (Michell 1976, 1978).

Beal et al. (1975a) reported that a negative relationship existed between the concentration of K in plasma and the flow rate of parotid saliva in sheep, with the resting rate of secretion dependent on the K concentration at any time. In general, however, the concentrations of K required to effect a response in saliva flow rate were higher (at 6-7 mmol/ℓ) than those observed in Experiment 8. The concentrations of Na and K in parotid saliva were found to be positively correlated with plasma concentrations (Beal et al. 1975a) but such a correlation was not observed here.

The effects on the results of leakage of fluid from the fistula

Problems arose in Experiment 8 from leakage of rumen fluid from the fistula and the possible effects of leakage on the quantity of digesta in the reticulo-rumen in all experiments needs consideration.

It was not possible to measure leakage when the cows were in feeding stalls (as opposed to digestibility stalls), but the leakage of 0-8 kg/cow/day that occurred in Experiment 8 may also have occurred in the feeding stalls on the days when digesta measurements were made.

Fluid lost through leakage would decrease the quantity of fluid in the reticulo-rumen and therefore decrease both the loss to the omasum and the quantity remaining 21 h after feeding. The effect on the quantity remaining at 21 h would be smaller than the actual amount lost through leakage, therefore a large difference between susceptibility groups in the quantity lost by leakage would have been required to substantially affect the quantity at 21 h in LS cows relative to HS cows.

Leakage from cows in digestibility stalls occurred mainly when the cows were lying down. When in feeding stalls for digesta measurements, the cows lay down during the period of 8-21 h and not during 0-8 h after feeding, which is when differences between susceptibility groups in the rate of digesta loss and in the quantity of digesta were apparent. Whether leakage of rumen fluid affected digesta measurements or quantity of digesta in HS cows relative to that in LS cows cannot be resolved from the data. The alternative to using fistulated cows is to dose orally with markers and estimate volume and dilution rate by sampling rumen fluid using a stomach tube, or to estimate dilution rate and retention times of orally dosed fluid and particulate markers using faecal sampling techniques. Estimates of the quantity of digesta in the reticulo-rumen by marker techniques are less precise than by direct measurement, but may enable more animals to be studied.

Reid (1965) has discussed the possible effects of the removal of digesta on reticulo-rumen function and salivation and has noted the difficulties of obtaining representative samples from large quantities of digesta. He found no apparent ill-effects on DM loss from the reticulo-rumen of the removal of digesta 5 times in 24 h, and the data obtained here indicated no major discrepancies between successive estimates of the quantity of digesta before feeding that could not be accounted for by losses of DM and fluid in samples.

Conclusions

Aspects of the digestive characteristics of cows differing in susceptibility were investigated, including:

1. Digestibility of hay and pasture diets.
2. The quantity of digesta in the reticulo-rumen before and at intervals after feeding when the cows were offered restricted levels of hay and pasture diets, including diets differing in maturity and in the ratio of K:Na.
3. Capacity of the reticulo-rumen.
4. The rumen fluid dilution rate and the osmolality and concentrations of Na and K in rumen fluid of cows when offered hay and pasture diets, including diets of high and low K:Na ratio.
5. The effect on fluid dilution rate and outflow of digesta from the reticulo-rumen of the concentration of Na in rumen fluid.
6. Excretion of minerals in urine and faeces.
7. Some aspects of the composition of blood and saliva.

The major finding was that the quantity of digesta and the digesta fill were lower in LS cows than in HS cows under some experimental conditions. The quantity of digesta in the reticulo-rumen may, therefore, be one of the factors contributing to the susceptibility of a cow to bloat and may also affect the day-to-day incidence of bloat in cows of known susceptibility.

Composition of the diet was considered to be the most likely factor influencing the results. In hindsight, insufficient attention was given to the composition of the diets, or even to an adequate description of their species content, to enable the identification of any factors that may have been involved in determining whether HS and LS cows differed in the quantity of digesta in the reticulo-rumen. However, these dietary factors are unlikely to be identified from simply measuring the concentrations of constituents in the herbage, and detailed investigation of the effects of specific diets or dietary constituents on the quantity, composition, and characteristics such as particle size, of digesta will be required. Such studies should include re-investigation of the effects of the quantity of digesta on the occurrence and severity of bloat in a greater number of cows than was originally used when the hypothesis of Cockrem _et al._ (1983) was proposed.

Susceptibility to bloat is a subjective classification, but was used objectively in the treatment of data. The standard errors of the differences between the means of the susceptibility groups indicated considerable variation existed between cows within each susceptibility group compared, for example, to those for comparisons of diets in Experiment 8. The extent to which this variation is accounted for by genetic factors relating to susceptibility cannot be determined from the data and will not be resolved while small numbers of animals are studied.

The mechanism by which the quantity of digesta was lower for LS cows than HS cows under some conditions was not established in these experiments. Further studies on the motility of the reticulo-rumen of HS and LS cows appear warranted. Two aspects of motility require investigation: to establish whether a difference between HS and LS cows in motility contributes to the difference in the quantity of digesta under some conditions, and to study the effects of the quantity of digesta on reticulo-rumen motility and on other aspects of reticulo-rumen function such as the production and composition of saliva.

These studies on aspects of the digestive characteristics of cows differing in susceptibility to bloat have indicated that HS and LS cows are similar in many respects. Cows of high and low susceptibility may show the greatest differences in digestive characteristics when offered bloat potent diets. Whether or not this is so should be experimentally established at any early stage in any extension of the research programme reported in this thesis.

Appendix 1 Information on cows with rumen fistulae.

Cow	Susc. group	Year born	Susc. grade	Breed[a]	Experiments	Date of fistulation
701	HS	1977	2.65	50	3-8	Dec 1981
705		1977	1.77	37	3,4	" "
908		1979	2.00	28	3-8	Feb 1983
909		1979	2.61	37	3-8	" "
007		1980	1.73	40	3-8	" "
729	LS	1977	0.52	25	3,4	Dec 1981
730		1977	0.64	50	3-8	" "
930		1979	0.41	68	3-8	Feb 1983
934		1979	0.37	50	3-8	" "
036		1980	0.15	34	3-8	" "

[a] Percent of Friesian; see Chapter 2.2.1 for derivation

Appendix 2a Preliminary investigation of addition of Cr EDTA to digesta and of site of sampling of digesta

Three methods of marker addition were compared:

(1) Injection of marker into digesta removed from the reticulo-rumen, prior to its return.

(2) Injection of marker into several sites within the rumen.

(3) Injection of marker into several sites within the rumen, after digesta had been removed and replaced.

Two procedures were used to sample the rumen fluid during the 6 h following marker addition.

(A) A sample from the reticulum.

(B) A composite sample from 3 sites around the rumen.

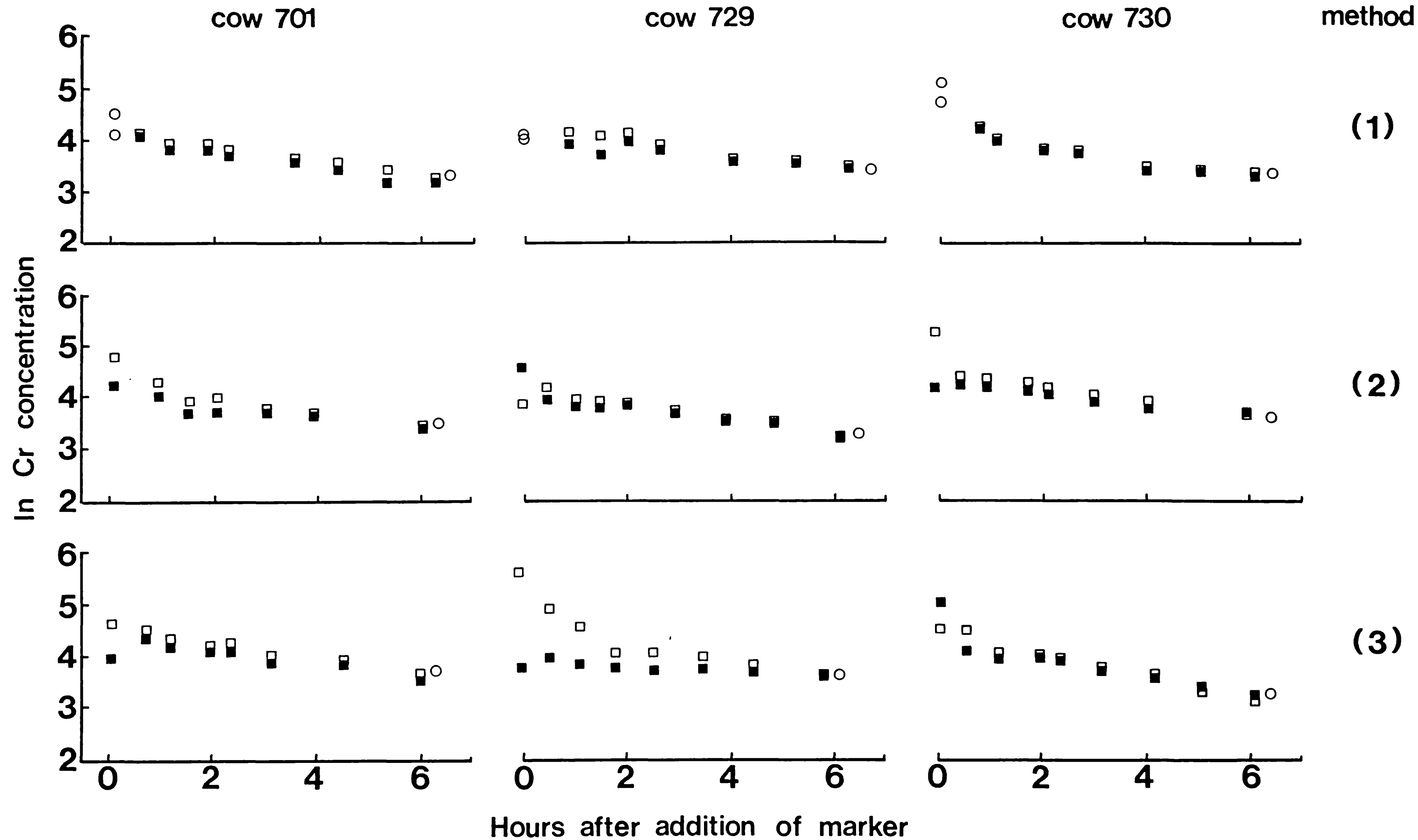
Each method of marker administration was carried out on each of 3 cows when offered a pasture diet. Food was removed prior to addition of marker. In method (1), rumen fluid was sampled from digesta after marker injection but before the digesta was returned to the reticulo-rumen. Digesta was removed, mixed and sampled for all cows 6 h after marker addition.

The ln Cr concentration was plotted against time (h) for each cow and each method (Appendix 2b). The graphs indicated:

(1) Concentrations of Cr in samples from bailed digesta (method (1)) immediately after marker addition showed that the hand-mixing of digesta and Cr may be incomplete.

(2) Concentration of Cr in samples from the reticulum were often lower than those from composite samples when the marker was added *in vivo*.

(3) At least 2 h was required for mixing of digesta with marker added *in vivo*, even when digesta had been mixed by prior removal.



Appendix 2b The relationship between ln Cr concentration and time (hours) for 3 cows and 3 methods of marker addition. Rumen fluid was sampled from the reticulum (■), rumen (□) or from digesta removed by bailing (○).

Appendix 3a Salivary Na:K ratios of HS and LS cows offered pasture when unsupplemented (weeks 1 and 2) or supplemented (week 3) with 30 g NaCl/day.

Susceptibility group	Cow	Week 1	Week 2	Week 3
HS	007	2.0	0.8	7.2
	701	1.5	1.6	5.5
	705	10.8	16.9	18.9
	908	4.6	3.2	18.7
	909	13.3	8.3	17.9
LS	036	10.0	0.9	1.6
	729	12.6	18.3	22.5
	730	14.4	7.5	15.9
	930	2.0	1.5	10.6
	934	2.9	1.1	9.8

Appendix 3b Na and K concentrations (mmol/ℓ) of rumen fluid from HS and LS cows offered pasture when unsupplemented (weeks 1 and 2) or supplemented (week 3) with 30 g NaCl/day.

	Week							
	1		2		3			
	HS	LS	HS	LS	HS	LS	sed[a]	sig.
Na								
0-8 h[b]	73.0	74.8	64.1	58.5	97.0	89.8	2.1	***
21 h	88.3	105.6	82.7	80.8	124.3	118.3	6.5	**
K								
0-8 h	60.1	55.9	67.9	59.5	44.8	49.2	1.5	***
21 h	43.6	33.8	58.3	56.5	22.6	27.9	5.6	*
Na + K								
0-8 h	133.1	130.7	132.0	118.0	141.8	139.0	2.5	***
21 h	131.9	139.4	141.0	137.3	146.9	146.2	2.6	*

[a] Standard error of the difference for the interaction between week and susceptibility group

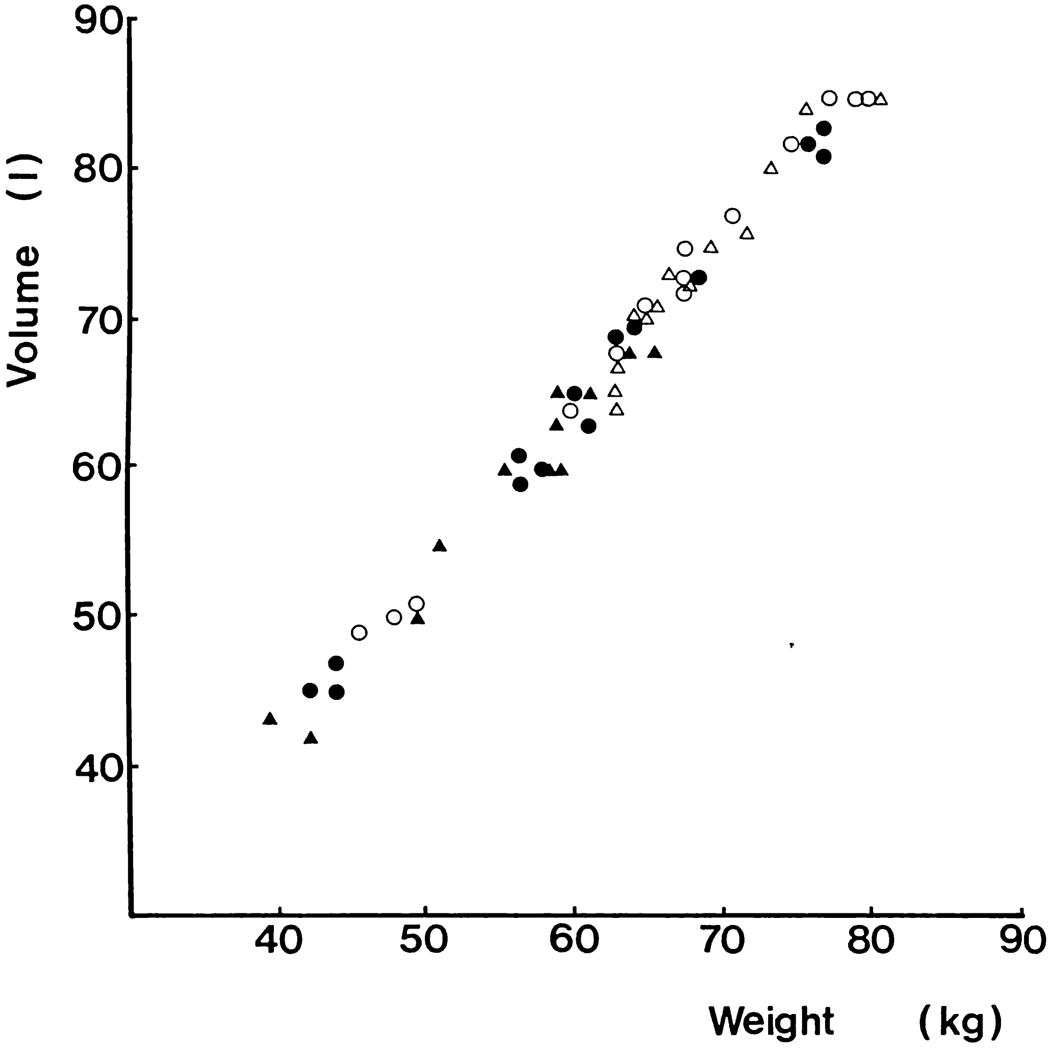
[b] Time after feeding period (h)

Appendix 3c Intakes (kg/cow) and quantities (kg) of fluid and DM removed at 0 and 21 h for HS and LS cows offered hay and pasture (Experiment 3).

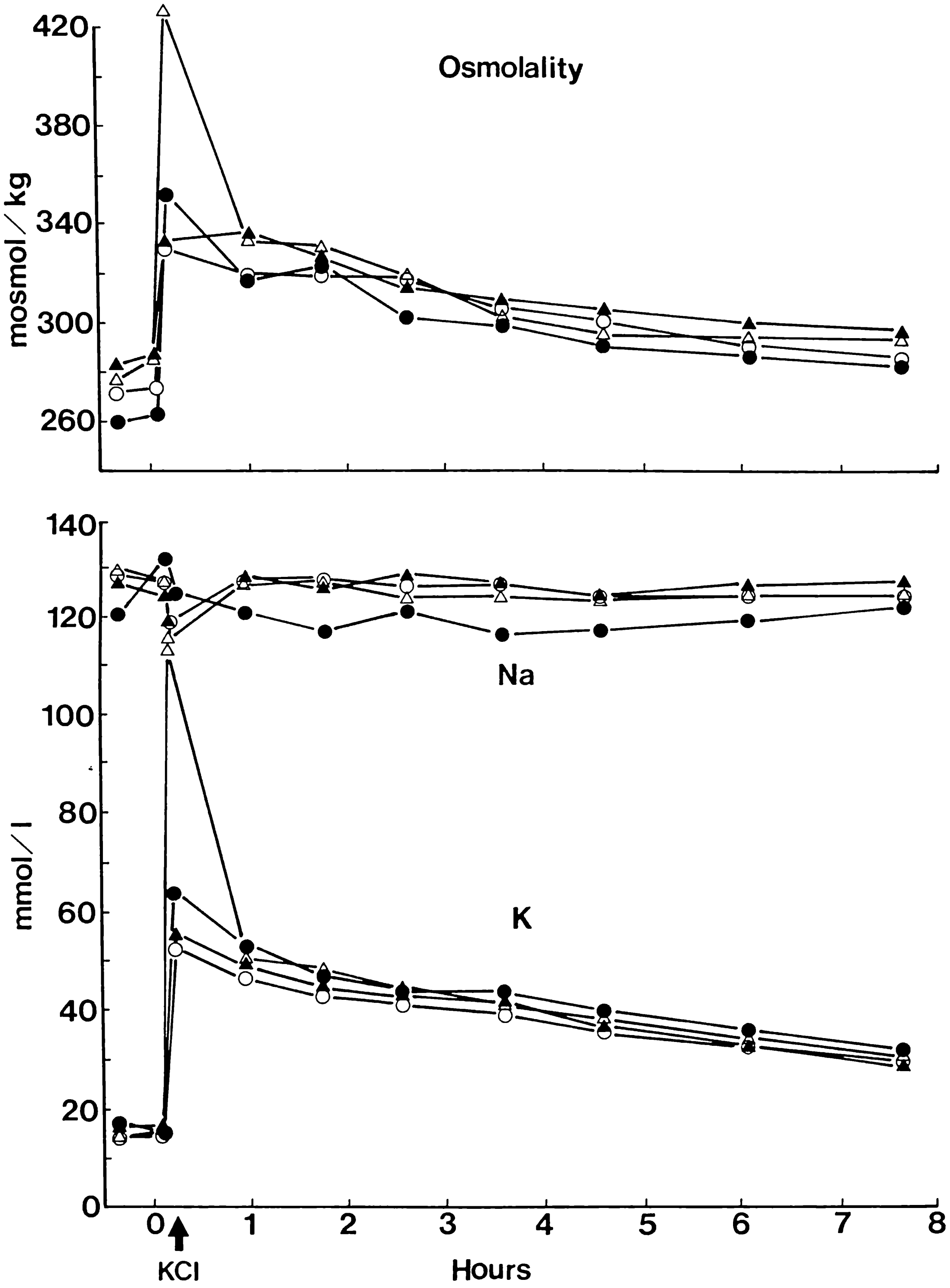
	Week							
	1		2		3			
	HS	LS	HS	LS	HS	LS	sed[a]	sig.
Hay								
Fluid								
Intake	20.1	21.2	19.5	19.4	22.6	22.2	4.2	ns
0 h	56.6	59.5	57.5	61.6	62.3	63.6	6.4	ns
21 h	35.2	35.4	37.5	38.0	38.8	37.9	2.5	ns
DM								
Intake	4.61	4.94	4.85	5.26	4.72	5.12	0.44	ns
0 h	7.78	8.22	7.99	8.36	8.30	8.62	0.71	ns
21 h	3.21	3.55	3.82	3.70	3.21	3.70	0.33	*
Pasture[b]								
Fluid								
Intake	22.4	29.0	22.8	35.4	17.5	19.0	6.1	ns
0 h	58.3	59.4	55.8	62.4	54.0	48.5	6.8	ns
21 h	37.2	29.9	35.3	28.5	38.6	28.2	3.7	ns
DM								
Intake	3.77	3.83	3.03	2.88	2.93	2.86	0.39	ns
0 h	6.05	6.53	5.64	5.93	5.28	5.36	0.77	ns
21 h	3.25	2.68	2.90	2.39	2.82	2.32	0.37	ns

[a] Standard error of the difference for the interaction between week and susceptibility group

[b] Unsupplemented weeks 1 and 2, supplemented week 3 with 30 g NaCl/day



Appendix 4 The relationship between the volume (ℓ) and the weight (kg) of digesta removed from the reticulo-rumen after feeding for HS (●,○) and LS (▲,△) cows offered hay (○,△) and pasture (●,▲). Volumes greater than 85 ℓ were unable to be measured.



Appendix 5a The effect of intraruminal addition of 5 ℓ of 0.48 M KCl solution on the osmolality and concentrations of Na and K in rumen fluid from 2 HS cows (●,○) and 2 LS cows (▲,△).

Appendix 5b The quantity (kg) of fluid and DM returned to the reticulo-rumen after addition of 5 ℓ of 0.48 M KCl solution, and subsequent losses (kg) over 1.5 and 7.5 h, for 2 HS and 2 LS cows.

	HS		LS	
Cow	908	909	930	934
Fluid				
Quantity	58.99	64.95	61.13	58.86
Loss 0-1.5	-0.36	1.31	-0.87	-0.71
1.5-7.5	6.05	5.57	6.56	8.48
0-7.5	5.69	6.88	5.69	7.17
DM				
Quantity	5.61	5.05	6.37	5.24
Loss 0-1.5	0.76	0.49	1.77	0.41
1.5-7.5	0.95	0.43	0.94	1.02
0-7.5	1.71	0.92	2.71	1.43

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