PLANT AND ANIMAL FACTORS
AFFECTING THE DIET SELECTION OF SHEEP

by

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DECLARATION

I declare that this thesis has been composed by myself. The experimental work and analyses were carried out by myself, with the assistance of other people as indicated in the acknowledgements. The work in this thesis has not been submitted for any other degree or qualification.
DEDICATION

To my mother for all that she has taught me

All things come of thee and of thine own
have we given thee.

1 CHRONICLES, 29:14.
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ABSTRACT

The purpose of this work was to test the ability of sheep to select between foods of different energy density (ED; MJ ME/kg food) a diet which meets their physiological needs, in a series of five experiments.

Experiment 1 used pregnant and contemporary non-pregnant ewes to test the hypothesis that sheep can select a diet which meets their needs for protein. Four foods were offered: foods A and B were of high ED and foods C and D were of low ED; foods A and C were of high crude protein (CP) concentration, foods B and D were of low CP concentration. The treatments included paired choices of foods A and B, and foods C and D, which were offered to the ewes. The results showed that when the ED of foods is high, pregnant ewes select a diet that reflects their enhanced protein requirements, in contrast with non pregnant contemporaries. When ED is low, priorities other than physiological state may motivate dietary choice. It is suggested that such a priority is the maintenance of a balanced rumen environment. Experiments 2 and 3 tested the effect of ED of foods offered as a choice on the diet selection of sheep and the relationship between the rumen environment and the diet selected from a pair of foods of different ED. The diet selected by the choice-fed sheep in Experiment 2 did not consist solely of the high ED food (H), but contained a substantial quantity of the low ED food (L). It was hypothesised that the diet selected by sheep from a choice of low and high ED foods is affected by the rumen conditions, specifically rumen pH and osmolality, and that sheep will alter their pattern of dietary choice to maintain optimal rumen conditions. In experiment 3, rumen infusion and sampling techniques were used to test whether hypertonic rumen infusates of acid, alkali or saline affect the diet selection of sheep offered a choice of foods H and L. Intake of food H was affected by increases in infusate osmolality, but intakes of L were not. Diet selection was not affected by rumen pH. The aim of experiment 4 was to test the effects on the diet selection of lambs of offering a low ED food in different physical forms (alfalfa, pelleted or long chop), high ED foods intended to create different rumen fermentation patterns
(barley-based; sugarbeet/barley-based), or the inclusion of sodium bicarbonate (\(\text{NaHCO}_3\); 0, 1, 2 & 4 % (w/w)) as a rumen 'buffering' agent. Although diet selection was not affected by \(\text{NaHCO}_3\), the physical form of the low ED foods and the fermentation characteristics of the high ED foods appeared to affect diet selection such that more of the low ED food was selected when it was pelleted than when it was in a long-chop form. Experiment 5 was designed to test whether the physical form of a low ED food, when offered as a choice with a high ED food, affects its selection by sheep, and to study the pattern of dietary choice as influenced by a challenge to existing rumen conditions. The results suggested that the diet selection pattern of sheep is maintained in response to a rumen challenge when the low ED food is pelleted, but not when it is unprocessed.

Taken together, the results presented show that sheep can adjust their dietary selections according to the nature of the rumen environment. The results are discussed in the context of short and long-term feeding strategies for sheep.
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Chapter One

Background
INTRODUCTION

Many animals inhabit an environment which contains a variety of foods. Of these foods few, if any can supply all the nutrients that are essential for the growth and development of animals in sufficient quantities, and many contain anti-nutritive factors (Westoby, 1974). Therefore animals, including ruminant species, have successfully developed dietary strategies that ensure they optimise the advantages to be gained from eating the foods available to them to achieve rapid growth and the production of viable offspring, whilst minimising any disadvantages, for example high levels of anti-nutritive factors, such as alkaloids and tannins (McDonald et al. 1988), that are associated with the foods available (Emmans, 1991).

Considerable evidence exists which shows that monogastrie farm livestock, as well as labororatory animals (for reviews see Rozin, 1976; Rose & Kyriazakis, 1991), can select an adequate diet from pairs of foods that differ only in the concentration of one nutrient, such as protein (chickens: Shariadmadari & Forbes, 1990; pigs: Kyriazakis et al. 1990; Kyriazakis, 1994). The diets selected by the animals in these experiments enabled them to grow rapidly, whilst avoiding an excessive intake of the nutrient under question. Kyriazakis & Emmans (1991) have demonstrated that the sex of pigs and their previous nutrition can have a marked effect on the choices made between foods of different crude protein concentration. Sheep offered foods that differ in crude protein content (Cropper, 1987; Hou, 1991; Kyriazakis & Oldham, 1993) are also able to make structured choices between the foods offered and therefore select a diet which meets their requirements for rapid growth.

The genotype of monogastrie animals (chickens: Shariadmadari & Forbes, (1990) and pigs: Kyriazakis et al. (1993)) also has an influence on their dietary choice, as animals of different breeds are able to select diets that are appropriate to the typical growth characteristics of their genotype. Arnold et al. (1981) have demonstrated that different breeds of sheep exhibit
preferences for different plant species when grazing; however no attempts have been made to relate the dietary choices made by sheep to the growth characteristics that are typical for their genotype.

It is recognised that the physiological state of an animal affects its requirement for energy, protein and other nutrients and some attempts have been made to systematically record these changes, for example Agricultural Research Council, (1980), (sheep) and Agricultural Research Council (1981) (pigs). Differences in physiological state can have an effect on the quantity of food consumed by animals (Weston, 1982; Kyriazakis, 1994). There are some recent studies of state-dependant diet selections in sheep, Newman et al. (1994) have demonstrated that fasted sheep select a lesser quantity of clover than non-fasted contemporaries. Kyriazakis et al. (1994) have shown that sheep with an endoparasitic load (the gastro-intestinal parasite, Trichostronglyus colubriformis) increase their selection of a high-protein food. However lactation apparently has no significant effect upon the dietary choice of grazing sheep (Parsons et al. 1994). Little else is known about the ability of sheep and other ruminants to alter their dietary choices according to their physiological state.

The aim of this chapter is to review existing literature that provides a physiological and behavioural background to the study of diet selection in ruminants. Throughout, the influence of food factors on diet selection will also be considered. Ingestive behaviour in ruminants differs from non-ruminant species because fermentative digestion has developed in conjunction with physiological mechanisms to utilise the end products of microbial activity (Bell, 1984). Thus an understanding of ingestive behaviour and ruminant physiology is needed when considering diet selection in ruminants.
Environment
1 Ambient temperature
2 Faecal contamination
3 Soil type
   mineral content
4 Available plants
   protein
   carbohydrate
   anti nutritive factors

Genotype
1 Growth characteristics
2 Specific appetites
3 Olfactory sense
4 Dental arcade width
5 Rumen capacity
6 Ability to utilise fibrous foods

DIET SELECTION

Physiological state
1 Sex
2 Previous nutrition
   level of fatness
   labile protein
3 Pregnancy/ lactation
4 Period since last meal
5 age

Previous history
1 Observed
   maternal
   non-maternal
   conspecific
2 'Trial and error'
3 Conditioned
   taste aversions

*Figure 1:1: Schematic representation of the factors which influence the dietary choices made by sheep*
MODELS OF INGESTIVE BEHAVIOUR

The ingestive behaviour of sheep and other ruminants is influenced by many factors (Milne, 1991); this is illustrated in Figure 1:1. The subsequent sections outline several theories that attempt to account for the diet selection of ruminants; each placing an emphasis on different aspects of their ingestive behaviour.

**Euphagia**

This theory is defined as the innate ability of animals to select a diet that is appropriate to their requirements. It was proposed from the work of Richter (1943) that livestock possess an innate ability to choose an appropriate diet. Pica has been described as evidence of the existence of nutrient-specific appetites, which are defined (Rozin, 1976) as an innate 'desire' to consume a specific nutrient, such as sodium to remedy a deficiency. Danford (1982) cites examples of cattle that have become deficient in phosphorus exhibiting nutritional wisdom by licking bones. Other more anecodal examples of specific appetites are known to the veterinary and medical professions (cf. Southgate, 1968). A specific appetite for sodium is the only one which is well documented (Rozin, 1976); calves will actively seek out sodium after they have developed a sodium deficiency (Sly & Bell, 1979).

It is unlikely that animals are equipped with a series of pre-wired recognition systems *ab utero* for each substance for which they can show specific appetites (Arnold, 1964; Rozin & Kalat, 1971). If such systems did exist, they would negate the ample evidence that ruminants and other animals are capable of forming associations between foods and the post-ingestive consequences of eating them (eg. Thorhallsdottir et al. 1990; Burritt & Provenza, 1991). In a review of the literature, Zahorik & Houpt (1977) proposed that, if animals solely use an innate knowledge of the foods around them when making dietary choices, this would imply that
ruminants that select poisonous plants such as yew and ragwort, have an 'inherent desire to commit suicide'. Several authors, including Owen (1992) and Provenza & Balph (1990) have proposed that it is unlikely that diet selection by ruminants can be considered solely as the sum of various specific and innate appetites.

Hedyphagia

The word 'hedyphagia' is derived from the Greek, *hedone*, which means pleasure; this model assumes that animals make dietary choices on a purely sensual basis. The selections made by sheep within the vertical plane of a sward generally demonstrated that ruminants prefer young rather than old tissue, leaf tissue to stem tissue (Bazely, 1990; Milne 1991). It is possible that sheep make such dietary choices through a reliance on their senses alone. Arnold (1966) determined that the senses of taste, smell or sight are all important in the process of making dietary choices in ruminants. The buccal cavity of ruminants contains a very large number of taste buds, and through these ruminants are able to discriminate between salty, bitter, sour and sweet tastes (Bell, 1959). There is some evolutionary benefit in showing a preference for sweet-tasting foods as these foods are frequently sources of energy and in avoiding bitter-tasting foods, which are frequently associated with the presence of toxins such as alkaloids and cyanogenic glycosides (Rozin & Vollmecke, 1986). However preferences for foods to which specific flavours have been added (Nolte & Provenza, 1992) are only sustained if the different flavours are associated with different post-ingestive consequences. Hedyphagia accounts for the ability of ruminants to discriminate between broad differences in dietary items. A limitation of this model is that it focuses on the effect of gustatory stimuli on dietary choice and it excludes the effect of post-ingestive stimuli on dietary choices (Provenza & Balph, 1990).
The concept of optimality is central to behavioural ecology. It assumes that natural selection promotes the survival of those genes that are associated with advantageous behavioural characteristics. MacArthur & Pianka (1966) presumed that large generalist herbivores and other animals follow an optimal foraging strategy when selecting a diet. Krebs & McCleery (1984) cite theoretical examples in which optimal foraging theory has been used to predict when animals will move from one area to another in order to maintain an optimal rate of energy intake. This aspect of optimal foraging theory is of direct relevance to the study of grazing animals such as cattle and sheep.

There is some evidence that is consistent with the hypothesis that sheep forage optimally (Kenney et al. 1984) when selecting a diet. However there is frequently a considerable difference (eg. Broom & Arnold, 1986; Cropper, 1987; Parsons et al. 1994) between the diet actually selected by large generalist herbivores, such as sheep and the diet it is predicted they should select according to optimal foraging theory. Constraints in the real world can prevent animals, especially large generalist herbivores, from selecting a diet that would be viewed as optimal (Malechek & Balph, 1987), with respect to its energy content. Even in the relatively controlled environment that is associated with a conventional feeding trial (Cropper, 1987), sheep do not select a diet that would be predicted from optimal foraging theory. Certainly the optimal foraging model appears to be more applicable to carnivores than herbivores, as the prey of carnivores does not vary greatly in quality unlike the plant material available to herbivores (Westoby, 1974).

Westoby (1974) attempted to account for the limitations of optimal foraging theory by proposing that large generalist herbivores do not seek to maximise their rate of energy intake solely, but instead seek to optimise the nutrient mix that is ingested. Belovsky (1978) has
extended this hypothesis by successfully employing digestive and nutrient constraints, such as gut capacity and requirements of nutrients such as sodium, to predict the feeding behaviour of animals. In addition, Westoby (1978) proposed that the existence of sampling behaviour is consistent with optimal foraging theory.

Sheep can learn to associate foods with the post-ingestive consequences of eating them (e.g. Thorhallsdottir et al. 1990), and that the subsequent diet selection by sheep indicate that they retain such information (Keogh & Lynch 1982; Thorhallsdottir et al. 1987). Classical optimal foraging theory appears to ignore this evidence. At present classical optimal foraging theory is useful as a tool, but does not appear to predict the dietary choices of ruminants in an accurate manner (Emmans & Kyriazakis, 1994) unless other constraints are included in the model.

**Morphophysiology**

Reference has already been made to the importance of digestive constraints on the diet selection by herbivores (Belovsky, 1978). There is considerable interest in the effect of animal factors, such as body size, on diet selection (e.g. Gordon & Illius, 1988; Illius, 1989). There are also several reviews of this aspect of diet selection (Milne, 1991; Illius & Gordon, 1990). In general large ruminants can utilise fibrous materials more effectively than smaller ruminants (Milne, 1991). This is due to the fact that larger ruminants have larger rumens which ensure that fibrous materials can be retained for extended periods. As a consequence smaller ruminants are obliged to select diets with high concentrations of cell contents rather than fibrous cell wall materials. Hofmann (1989) has used such morphological differences to classify herbivores into concentrate and roughage eaters. Within the same species, older animals are better equipped to utilise lower quality roughages than younger ones (Weston et al. 1989) as they are larger. Gordon & Illius (1988) have shown that the size of the dental arcade of a grazing animal is of great importance, its width determines the bite size of the animal and
its ability to make precise selection between plants. Sheep have a relatively narrow muzzle, which enables them to select plant parts with great precision. L'Hullier et al. (1984) have found that sheep are able to select leaf material predominately from a sward, where such material is less than 5 cm above the ground.

There is ample evidence that the broad morphophysiological attributes of animals do affect diet selection. However individual animals differ in their ability to tolerate materials such as urea (Kyriazakis & Oldham, 1993) and anti-nutritive factors (Provenza & Balph, 1990). It is likely that such differences between individual animals may affect diet selection (Provenza & Balph 1990), and that this may account for some of the variation that is inherent to all diet selection experiments (eg. Kyriazakis & Oldham, 1993).

*Learning through consequences*

This model is based on the assumption that dietary preferences develop through an interaction of positive and negative post-ingestive consequences of foods and the pre-ingestive experiences of the animal, which may be social or through trial and error. A precept of the learning model is that animals have an inherent ability to learn about foods and that this ability is a product of natural selection (Provenza & Balph, 1990).

Sheep have a conservative approach toward new foods (Chapple & Lynch, 1986). They will ingest small amounts of new foods and will gradually increase the quantity consumed if no adverse effects ensue (Chapple & Lynch, 1986). The effect of post-ingestive consequences and social models on diet selection have been studied systematically (Burritt & Provenza, 1989; Thorhallsdottir et al. 1990; Provenza et al. 1993a) by pairing foods with Lithium chloride (LiCl). This acts as a non-lethal gastro-intestinal poison; its effects are dose-related, ranging from slight discomfort to diarrhoea and vomiting (Lynch et al. 1992). The use of this
technique has shown that lambs can demonstrate conditioned taste aversions when allowed to watch and preferably participate with adult role models or contemporaries (Thorhallsdottir et al. 1987; Thorhallsdottir et al. 1990; Mirza & Provenza, 1992) that have developed aversions to the food under investigation. These aversions are stronger if the adult sheep is the mother (Thorhallsdottir et al. 1990). Burritt & Provenza (1989) have demonstrated that lambs are also capable of learning such associations by trial and error, and they do not automatically select the same diet as their mothers (Provenza et al. 1993a).

Zahorik & Houpt (1977) did not give much credence to the proposals that a 'learning by consequence' model could account for the dietary choices of ruminants. However at that time little evidence existed which illustrated that ruminant animals can display long-delay learning. Since then ample evidence has been produced to show that sheep can remember foods that are associated with aversive consequences (Burritt & Provenza, 1991; Thorhallsdottir et al. 1987) through the use of LiCl. However there are few examples of sheep remembering foods that are associated with positive ingestive consequences (Keogh & Lynch, 1982; Green et al. 1984).

Conditioned aversions to foods paired with LiCl can persist for up to 60 days (Thorhallsdottir et al. 1987) and there is evidence that sheep can remember foods that could be associated with positive ingestive consequences for an extended period (Green et al. 1984). However, it is unlikely that once animals have learnt to associate a food with the consequences that result from its ingestion, these linkages would absolutely determine subsequent dietary choices. Making immutable responses to foods would not be to the advantage of the animals concerned, as this would lessen their ability to cope with their changing requirements and changes in the foods available. Indeed, sheep which have become averse to LiCl-paired foods do not avoid it absolutely (Thorhallsdottir et al. 1987) as sheep continue to sample the LiCl-paired food. Ruminants and other herbivores appear to need to continuously sample the foods that are available to them to detect subtle chemical changes in these foods (Westoby, 1974;
Westoby, 1978). This aspect of ingestive behaviour is retained even when the animals live in an relatively constant environment (Kyriazakis & Oldham, 1993).

It should be noted that the literature which supports the learning model relies heavily on the use of LiCl. This may be considered an extreme medium, as sheep can be made averse to foods paired with LiCl, at a dosage as low as 150 mg/kg body weight (duToit et al. 1991). In addition, as sheep commonly take 10-40 000 bites per day, the system required to process all the information that is gained must be complex (Milne, 1991).

All the diet selection theories that have been outlined above can account for aspects of the ingestive behaviour of animals. However, it is unlikely that one model alone can be used to account for the feeding behaviour of sheep and other animals in the complex environment in which they inhabit. A greater understanding of the diet selection made by sheep and other animals would be gained from an integration of these theories.

RUMEN ENVIRONMENT AND ITS EFFECT ON FOOD INTAKE AND DIET SELECTION.

Whichever strategies influence the dietary choices of an animal, these must operate within the framework of its physiology, specifically its gastro-intestinal physiology (Penry, 1993). Amongst monogastric animals, the pattern of nutrient absorption can be safely predicted from an accurate knowledge of the nutrients present in the foods in their diet. A study of the diet selection of sheep should not neglect the central role of the rumen in food intake and its possible role in the dietary choices of sheep. The subsequent section outlines literature which relates to the microbial activity within the reticulo-rumen. Secondly some consideration will be given to the effect of environmental conditions on microbial activity and the sheep itself. This
review of the literature is by no means an extensive one, since it relates to those aspects of the rumen environment which are considered in the experiments to be discussed later. For more extensive reviews see Carter & Grovum (1990a) and Forbes & Barrio (1992).

Microbial activity within the reticulo-rumen

Ruminants have evolved a capacious set of stomachs. Food which passes through these stomachs is changed markedly, due to the action of resident symbiotic organisms (Stevens, 1988). The microflora within the rumen possess the ability to digest cellulose and hemicellulose. The volatile fatty acids (VFAs) produced as end-products of this microbial activity can supply up to 70% of the energy absorbed by ruminant animals (Blaxter, 1967).

Microbial fermentation

Both the pattern of VFAs produced and their total concentrations are influenced greatly by the source of dietary carbohydrate (Van Soest, 1982; Sutton et al. 1986). At present there are no reliable means of predicting accurately the composition of VFAs produced in the rumen on the basis of diet composition (Beever, 1993). However several generally accepted associations can be stated that relate the composition of foods to the pattern of VFAs produced. Acetic acid is the predominant VFA that is produced from the fermentation of foods with high concentrations of cellulose and hemicellulose (Van Soest, 1982). When starchy materials, such as cereals are included in the diet of ruminants, the production of propionate increases at the expense of acetate (Sutton et al. 1986).

Microbial protein synthesis

Rumen micro-organisms can degrade dietary protein, yielding peptides, amino acids and ammonia. The peptides and ammonia that are produced from these catabolic processes, together with dietary non-protein nitrogen and urea can be used by the resident micro-organisms to synthesize protein (for a review see Lewis & Hill, 1983). Microbial protein is the
major source of amino-acids for ruminants (Asplund, 1986; Beever, 1993). The importance of energy and protein interactions within the rumen are well recognised (Beever, 1993; Sinclair et al. 1993). Oldham et al. (1977) recognised that imbalances between the rate of degradation of nitrogenous compounds and the rate at which dietary carbohydrates are fermented have a substantial influence upon the amount of nutrients made available to a ruminant from its food.

*Conditions within the rumen*

The environment in which rumen bacteria, protozoa and fungi reside is relatively constant for a given diet. The rumen is highly anaerobic and its temperature remains relatively static (38-42°C) (Theodorou & France, 1993). Rumen pH is generally maintained within the range 6.0-7.0; however, in certain circumstances rumen pH can drop to 5.5, following the consumption of large quantities of grain and other readily fermented foods (Counette et al. 1979). The VFAs that are produced by the fermentative actions of the resident bacteria are buffered by the bicarbonate and phosphate salts that are contained within saliva that is produced in copious quantities by ruminants (Turner & Hodgett, 1955).

*The effect of rumen pH on rumen micro organisms and ruminant animals*

The maintenance of rumen pH is of great importance to the resident micro-organisms and is of direct and indirect importance to the host ruminant (Carter & Grovum, 1990a). When the pH of the rumen is lowered, a large proportion of the energy generated by fermentation is used to maintain the bacterial cells (Russel & Hespell, 1981). An increase in bacterial maintenance energy is of indirect significance to the ruminant as host animal, as this would reduce the yield of microbial protein (Hespell & Bryant, 1979; Russel & Strobel, 1993).

Bacteria show different abilities to withstand the effect of a decline in rumen pH (Russel & Dombrowski, 1980); this affects both the pattern of VFAs produced and the total concentration of VFAs generated. The acetate: propionate ratio is reduced at low pH (Beauchemin & Buchanan-Smith, 1989), as a consequence of the reduced activity of cellulytic
bacteria (Mould & Orskov, 1984). When rumen pH is low, amylolytic bacteria continue to flourish (Russel & Dombrowski 1980), although at lower levels of efficiency, and the production of lactic acid is increased (Erflle et al. 1982; Van Soest et al. 1991).

Rumen motility is reduced markedly if the rumen pH is low (Critchlow, 1988); this is partially a direct effect of the increased concentration of H⁺ ions and also an indirect effect of elevated concentration of lactic acid (Smith et al. 1979). Rumen papillae can become elongated and keratotic at low rumen pH (Block & Shellenberger, 1980). In extremis the integrity of the rumen epithelia itself may be compromised (Dirksen, 1970).

The effect of osmotic pressure of the rumen liquor on the rumen micro-organisms and the ruminant animal

The osmotic pressure of the rumen liquor is maintained within certain limits (Carter & Grovum, 1990a) through the production of saliva and through the influx of water across the rumen epithelia (Lopez et al. 1994). There is some evidence that cellulolysis is reduced at high osmolalities (Bergen, 1972); generally Gram-negative bacteria, many of which have cellulytic activities (Ketchum, 1988), are more sensitive to high rumen osmolalities than Gram-positive bacteria (Carter & Grovum, 1990a). Time spent ruminating is reduced when rumen osmolality is increased (Carter & Grovum, 1990a). Recently, Lopez et al. (1994) have demonstrated that the absorption of VFAs is reduced at high rumen osmotic pressures and that substantial shedding of epithelial tissues into the rumen can occur.

ATTRIBUTES OF FOOD AND RUMINAL METABOLISM THAT AFFECT FOOD INTAKE AND DIET SELECTION

The reticulo-rumen and microbial activity therein is crucial to the metabolism of the host ruminant (Theodorou & France, 1993). Many of the reviews of literature on food intake
regulation in ruminants give a central role to the activity of the rumen and the products that are synthesised (Weston & Poppi, 1987; Grovum, 1987; Forbes & Barrio, 1992). Some evidence already exists which is consistent with the view that indicates that the diet selected by ruminants is affected by the rumen environment (Cropper, 1987; Engku Azahan & Forbes, 1992; Kyriazakis & Oldham, 1993).

The effect of physical attributes of foods on intake and diet selection.

There is considerable evidence that the food intake by ruminants on roughage diets is limited by physiological factors such as digesta loads (for reviews see Van Soest (1982) and Forbes (1986)). The experiments of Conrad et al. (1964) have demonstrated that cows can alter their food intake according to the energy content of the food offered, if its digestibility is above a set point. Owen et al. (1969) have also found that lambs can compensate for the energy dilution of foods offered. The site of the point of inflexion in the relationship between dry matter digestibility and food intake depends on many factors such as rumen capacity (Weston et al. 1989).

Kenney et al. (1984) have demonstrated that the physical attributes of foods can affect the dietary choice by sheep, as sheep show a decided preference for foods which can be eaten with greater rapidity, when offered a two-way choice. However sheep do not show an absolute preference for foods of low bulk density (Cropper, 1987). Cropper (1987) suggested that one of the factors that may influence the dietary choice of sheep is the 'desire' to ensure the rumen environment remains in a fit and adaptive state. The results of Parsons et al. (1994) are consistent with this suggestion. Sheep which were offered a choice between foods which differed in energy density (ED) included a quantity of the low ED food in the diet selected, possibly because low ED foods are frequently those which stimulate chewing activity (eating + rumination) (Van Soest et al. 1991). Eating and rumination activities promote the
production of saliva (Balch, 1971; Woodford & Murphy, 1988). The significance of saliva as a buffering agent and diluent has already been alluded to (Carter & Grovum, 1990a). Balch (1971) and Sudweeks et al. (1981) recognised the physiological significance of chewing behaviour, and have proposed roughage value indices, which use time spent chewing as a means of classifying fibrous foods. Lynch et al. (1992) have proposed that the provision of materials which stimulate rumination is crucial as it enables sheep to satisfy a behavioural need. Frequently sheep appear to satisfy this need by activities such as chewing slats and wool (Cooper et al. 1994), if the foods available do not supply fibrous materials.

Effects of volatile fatty acids on food intake and diet selection

A great deal of attention has been given to the effect of VFAs on the control of food intake in ruminants, given their importance in the energy balance of ruminants (Forbes & Barrio, 1992). Intra ruminal infusions of the salts of acetate, propionate and butyrate can all have a depressive effect on food intake (Baile & Mayer, 1969), although the effect of infusing acetate and propionate is more marked. In general, more emphasis has been given to the effect of propionate and acetate on food intake, as they are produced and absorbed in greater quantities than butyrate (Forbes & Barrio, 1992). At present there does not appear to be any evidence in the literature which relates the pattern of VFAs produced in the rumen to the diet selected by ruminants.

Engku Azahan & Forbes (1992) have studied the influence of ruminal infusion of sodium acetate treatments on the diet selection made by sheep given a choice between hay and concentrate foods. This work indicated that infusions of sodium acetate tend to depress the intake of concentrate foods to a greater extent than infusions of sodium chloride.
The effect of rumen pH and osmolality on food intake and diet selection

The study of the specific effect of rumen pH on food intake can be difficult because it is not possible to manipulate pH without altering other aspects of the rumen environment, for example osmolality and various anion populations (Bhattacharya & Warner, 1967). However it is generally accepted (Counette et al. 1979; Forbes & Barrio, 1992) that the rapid ingestion of carbohydrates that can be readily fermented has the effect of causing rumen pH to drop rapidly. This is frequently associated with a depression in food intake.

Engku Azahan & Forbes (1992) appear to be the only authors who have considered the effect of rumen acidity and osmolality on the diet selection of sheep. When offered a choice between slowly fermented food (hay) and more rapidly fermented food (barley/grass-based), the sheep maintained their intake of hay, but reduced their intake of the barley/grass-based food when hypertonic solutions were infused into the rumen (Engku Azahan & Forbes, 1992). The effect of rumen pH on food intake may be mediated via receptors in the reticulum that have been identified by Critchlow (1988); the activity of these receptors is increased when the pH of the bathing medium is reduced. However Forbes & Barrio (1992) have suggested that the effect of rumen pH is more indirect, and may be mediated via effects on rumen motility, for example.

Food intake can be depressed by elevating rumen osmolality (Ternouth & Beattie, 1971; Carter & Grovum, 1990b). In a review of the factors controlling food intake, Grovum (1987) ascribed many of the effects of intra ruminal infusions of VFAs to other osmotic effects, citing the studies of Ternouth & Beattie (1971) and Grovum & Bignell (1989) in support of this hypothesis. Carter & Grovum (1990a) have found that sheep respond very promptly to the administration of a hypertonic rumen infusion by reducing their food intake. Carter & Grovum (1990a) proposed that this indicates the presence of osmoreceptors in the rumen. However
those which have been identified at present are only stimulated by osmotic pressures that exceed the physiological range (Harding & Leek, 1972).

*The effect of nitrogenous compounds on food intake and diet selection*

High dietary levels of non-protein nitrogen (NPN) (ie. ammonia and amines) can depress food intake and can alter the meal pattern of sheep (Fenderson & Bergen, 1976), especially when the level of dietary true protein is low. The direct infusion of urea into the rumen can also depress intake (Conrad *et al.* 1977). The depressive effect of high levels of NPN on food intake is of particular importance when the available food is silage (Buchanan-Smith & Phillip, 1986).

Sheep that are given a choice between foods of different crude protein concentration are able to select a diet which meets their requirements for rapid growth (Cropper, 1987; Hou, 1991; Kyriazakis & Oldham, 1993). However, when one of the foods offered is loaded with urea (5% w/w), the diet selected by the sheep suggests that they will avoid consuming high levels of NPN, if possible (Kyriazakis & Oldham, 1993). Following such a dietary strategy would reduce imbalances in the supply of nitrogen and the release of energy from fermentation, thus promoting high levels of microbial protein synthesis (Sinclair *et al.* 1993).

**SUMMARY**

From this review of literature, it is evident that the diet selected by ruminants is shaped by their morphology, prior experiences and innate preferences. There is some evidence that the diet selected by ruminants is also affected by animal factors such as their physiological state. This thesis has two objectives, the first being to give further consideration to the effect of
physiological state on diet selection. Since many of the factors that can have an influence on food intake are related to the rumen environment and the metabolism therein, the second objective is to study the effect of the rumen environment on the dietary choice of sheep. The five experiments reported in this thesis were designed to address these objectives.
Chapter Two

The effect of late pregnancy on the diet selections made by ewes.¹

THE EFFECT OF LATE PREGNANCY ON THE DIET SELECTIONS MADE BY EWES.

To test the hypothesis that sheep select a diet that meets their physiological needs (imposed by late pregnancy), four foods were formulated of either a high metabolisable energy (ME) concentration (A, B; 12 MJ/kg dry matter (DM)) or a low concentration (C, D; 9 MJ/kg DM). The crude protein (CP) concentrations of A and C were high (279 and 191 g/kg DM) relative to B and D (105 and 95 g/kg DM); the ratios of metabolisable protein: ME were 12 g/MJ for foods A and C and 4g/MJ for foods B and D. Twin-bearing and non-pregnant Greyface ewes of similar body condition were offered ad libitum, A, B and C as single foods and paired choices (A with B) and (C with D). Replication was a minimum of four ewes for single foods and six for the choices.

Food intakes by pregnant and non-pregnant ewes were similar until parturition was imminent, when the intakes of the pregnant ewes declined rapidly. The majority of pregnant ewes carried twin foetuses to term without problems. When the ME concentration of the food choice was high (A/B), pregnant ewes selected a greater proportion (P<0.05) of the food of high CP concentration than non-pregnant ewes: pregnant, 0.57 (se 0.074); non-pregnant,0.28 (se 0.105). By contrast, when ewes were offered a choice between foods of low ME concentration diet selection was unaffected by state: proportion of the higher CP food, C selected were: 0.70 (se 0.070) and 0.79 (se 0.069) for pregnant and non-pregnant ewes respectively. These results show that when the dietary ME concentration of foods given is high, pregnant ewes select a diet that reflects their enhanced demand for protein in contrast with non-pregnant contemporaries. However, when the ME of the foods is low, priorities other than physiological state may motivate their diet selection. It is suggested that such a priority is the maintenance of a balanced rumen environment.
INTRODUCTION

Sheep are able to select a diet from a pair of appropriate foods which meets their requirements for protein (Kyriazakis & Oldham, 1993). Most previous work on diet selection in sheep (Cropper, 1987; Hou, 1991; Kyriazakis & Oldham, 1993) has concentrated on testing the abilities of growing lambs to make selections for an appropriate concentration of crude protein (N x 6.25); less consideration has been being given to other physiological states. This paper reports work with ewes in the last two months of pregnancy.

During the last two months of pregnancy, the foetal lamb gains 80% of its birth weight (Robinson, 1977). In addition specific maternal tissues, such as the udder, develop and colostrum is synthesized (Russel, 1984). The requirements of pregnant ewes for protein and energy increase rapidly over the last two months prior to lambing in order to supply the growing needs for the foetus and maternal tissues (Agricultural Research Council, 1980). During this period the ewe must also supply the increasing glucose requirement of the foetus. Silver (1976) showed that the uterus of the pregnant ewe accounts for up to 70% of the glucose turnover in ewes. Forty to fifty per cent of the glucose taken up by the uterus is used directly by the foetus. Pregnant ewes are able to increase the rate of gluconeogenesis when food is available ad libitum by increasing their rate of food intake. When offered a fixed ration, pregnant ewes are also able to increase the rate of gluconeogenesis by directing a greater proportion of glucogenic precursors toward gluconeogenesis rather than an alternative fate (MacRae & Egan, 1983). Wilson et al. (1983) found that the glucogenic contribution of propionate increases during pregnancy. These substantial changes in demand for amino acids and glucose during pregnancy might suggest that, given the opportunity, ewes would adjust their selection between available foods to meet a changing demand.

Only one previous diet selection experiment using pregnant ewes is known to us (Gordon & Tribe, 1951). Many of the ewes in that experiment either died or did not lamb successfully and
it was concluded that the ewes did not show 'nutritional wisdom'. However subsequent diet selection experiments (Kyriazakis, 1989) have suggested that it is likely that the methodology used in that experiment was flawed.

The first objective of the work reported here was to test whether late pregnancy has an influence on the ability of ewes to select a diet from a pair of foods which enables them to meet their increasing requirements for energy and protein. The second objective was to consider whether selection for protein, by ewes in late pregnancy, is affected by the availability of non-amino acid glucogenic precursors. Foods designed to promote high or low rates of propionate production in the rumen were used to achieve different rates of supply of non-amino acid glucogenic precursors.

MATERIALS AND METHODS

Animals and Housing

Thirty-four pregnant and thirty non-pregnant Greyface (Border Leicester x Scottish Blackface) ewes were housed on 16 January 1992 in individual pens with slatted wooden floors. The housing has been described previously by Kyriazakis & Oldham (1993). All the ewes were scanned at housing to determine whether they were pregnant or not and the number of foetuses carried. Only ewes that were assessed to be twin-bearing or non-pregnant were used in the experiment. All pregnant and twenty non-pregnant ewes were drawn from the SAC Edinburgh flock; it was necessary to buy in a further ten non-pregnant ewes. At housing the pregnant ewes had a mean live-weight of 70.0 kg (sd 9.58) and a mean body condition score of 2.75 (sd 0.25) (on a scale of 0-5 , Russel et al. 1969). The pregnant ewes lambed 8 weeks (sd 1.5 days) after housing. The mean liveweight of the non-pregnant ewes that were drawn from the SAC Edinburgh flock was 70.5 kg (sd 7.91) with a condition score of 3.0 (sd 0.59)
at housing, the non-pregnant ewes that were bought in had a mean live weight of 62.3 kg (sd 9.94) and a condition score of 2.75 (sd 0.25).

Each pen measured 1.29 x 2.06 m and contained one or two metal troughs (according to the experimental treatment) and a bucket for fresh water. The pens with two troughs were chosen randomly within the shed. No artificial light-dark regime was used and the ambient temperature ranged from a mean daily minimum of 1.1 °C (sd 1.8) to a mean daily maximum of 8.6 °C (sd 2.8).

**Foods**

Four foods, A, B, C and D, were formulated and made into pellets. The formulation of the foods and their chemical composition are given in Table 2:1. Foods A and B were designed to have a high metabolisable energy (ME) concentration relative to foods C and D. The crude protein (CP) concentrations of A and C were high relative to B and D. Foods A and C were designed to have the same high calculated ratio of metabolisable protein (MP; Agricultural and Food Research Council, 1992): metabolisable energy (11.0 g MP/ MJ ME) to ensure that the selection made by the ewes offered a choice of two foods (choice-fed) could be compared on the same basis. For similar reasons, the MP: ME ratio of foods B and D were similar but lower (4.0 g MP/ MJ ME).

Foods A and B were formulated so that the glucose requirement of both ewe and foetus might be expected to be met predominantly via non amino-acid precursors such as propionate. To this end, foods A and B included a high proportion of starchy foods which would be expected to yield a rumen fermentation pattern characterised by a high proportion of propionate (Sutton et al. 1986). It was intended that the supply of non amino-acid precursors alone from foods C and D would be insufficient to supply glucose requirements and so glucogenic amino acids would have to be utilised. To achieve this, a high proportion of sugar beet feed was included in foods C and D to promote the production of acetate within the rumen at the expense of propionate.
Table 2:1: Ingredient and chemical composition of the experimental foods

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatfeed</td>
<td>125</td>
<td>83.8</td>
<td>487.0</td>
<td>458.2</td>
</tr>
<tr>
<td>Barley</td>
<td>496.2</td>
<td>597.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fishmeal/soya 1:3</td>
<td>312.1</td>
<td>-</td>
<td>216.0</td>
<td>-</td>
</tr>
<tr>
<td>Unmolassed sugar beet</td>
<td>-</td>
<td>-</td>
<td>231.0</td>
<td>476.4</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>-</td>
<td>246.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Limestone flour</td>
<td>-</td>
<td>4.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>-</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>-</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>6.6</td>
<td>-</td>
<td>6.6</td>
<td>-</td>
</tr>
<tr>
<td>Salt</td>
<td>5.6</td>
<td>-</td>
<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.2</td>
</tr>
<tr>
<td>CMS 20</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Calcined magnesite</td>
<td>2.5</td>
<td>3.2</td>
<td>3.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Optivite-ewe/lamb</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Analytical values (g/kg DM), unless otherwise stated.

| Dry matter | 842 | 850 | 833 | 862 |
| ME (MJ/kg DM)
| 12.0 | 12.0| 9.0 | 9.0 |
| fME (MJ/kg DM)
| 10.8| 11.3| 8.3 | 8.5 |
| Crude protein | 279 | 105| 191 | 95 |
| eRDP
| 178 | 77| 107 | 46 |
| eRDP/ME ratio | 16.48| 6.81| 12.89| 5.41|
| Neutral detergent fibre | 239.4| 217.7| 491.2| 537.5|
| Ash          | 73.0| 57| 76 | 79 |
| Calcium      | 8.8 | 5.8| 8.9 | 10.5 |
| Phosphorus   | 8.0 | 4.2| 4.3 | 4.4 |

Estimated production (g/kg DM)

| Microbial protein | 73 | 43 | 56 | 24 |
| Metabolisable protein (MP) | 140 | 48 | 108 | 40 |
| Ratio of MP/ME | 11.7| 4.0| 12.0| 4.4 |
| Effective rumen fermentable protein (eRDP) | 73 | 43 | 56 | 24 |
| Metabolisable protein (MP) | 140 | 48 | 108 | 40 |
| Ratio of MP/ME | 11.7| 4.0| 12.0| 4.4 |

a ME metabolisable energy, calculated from food tables.
b Values calculated using the Metabolisable protein system (AFRC, 1992); assuming a rumen outflow rate of 0.05 d⁻¹, and standard values for degradability coefficients.
c fME fermentable ME as defined in AFRC (1992).
d Effective rumen degradable protein (eRDP).

All four foods contained minerals and vitamins in quantities that were sufficient to meet the needs of the ewes (ARC, 1980).
Design

The ewes were randomly allocated to five feeding treatments: free and continuous access to a single food (A, B and C; single fed) or a free and continuous choice between foods (choice fed; A and B (A/B) or foods C and D (C/D)). The number of ewes allocated to each treatment is given in Table 2:2. A greater number of pregnant ewes were allocated to the food choice A/B so that any differences in diet selection would be distinct. In addition food D was offered as a single food to five non-pregnant ewes; it was not offered to pregnant ewes because it was judged, on ethical grounds that the low ME and CP content of the food would put those ewes at too high a risk of developing pregnancy toxaemia to be acceptable.

Table 2:2: Number of ewes allocated to each feeding treatment with numbers generating data

<table>
<thead>
<tr>
<th></th>
<th>Pregnant ewes (n)</th>
<th>Non-pregnant ewes (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allocated</td>
<td>Generating data</td>
</tr>
<tr>
<td></td>
<td>Allocated</td>
<td>Generating data</td>
</tr>
<tr>
<td>Single feeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>-5</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>Da</td>
<td>-</td>
<td>-5</td>
</tr>
</tbody>
</table>

a No pregnant ewes were allocated to this treatment because the mortality would have been high.

Directly after housing, the ewes allocated to the food choice treatments were given a training period of twelve days during which the foods were introduced to the ewes in an alternating pattern. In this period all the ewes were offered a decreasing quantity of hay and the quantity of the experimental foods offered was increased to *ad libitum*; this was to enable the digestive tract of the ewes to adapt to the new foods. Training was completed during the (predicted) 7th and 8th weeks prior to the time that the pregnant ewes were expected to start lambing; after the training period all the ewes were offered *ad libitum* the single food or food choice to which
they had been allocated. The non-pregnant ewes were introduced to the treatments at the same stage so that their intakes and selections were contemporary with the pregnant ewes.

Training periods of this type are an important part of choicefeeding experiments (Kyriazakis, 1989; Kyriazakis & Oldham, 1993), as they enable the animal to gain experience of the foods to be offered as a choice and to form associations about the foods. The dietary treatments and positions of the troughs for the ewes offered a food choice were randomised across the shed but were not changed during the experiment, as it has been adequately demonstrated that sheep do not make diet selections solely on the basis of food position (Cropper, 1987; Hou, 1991).

After lambing the ewes and their lambs were removed from the experiment. The experiment was designed to study diet selection in pregnant sheep during the last 6 weeks of pregnancy. The non-pregnant ewes continued on the experiment until all the pregnant ewes had lambed.

**Management and Measurements**

The ewes were offered fresh food twice daily (morning and afternoon) to minimise spillage; food refusals were weighed daily and discarded. Approximately 150g of Hay was offered to all ewes daily to minimise the risk of rumen acidosis developing. This was placed in bins separate from the pelleted foods. Weekly samples of the four foods were taken for dry matter determinations and chemical analyses were conducted on these samples.

The live weights of the ewes were measured on one day each week during the afternoon. On the same occasions the body condition scores of the ewes were assessed (to an accuracy of an eighth) (Russel et al. 1969). The birth weights of the lambs were measured within four hours of lambing and the live-weights of the ewes were taken on the morning after lambing. To monitor the metabolic state of the ewes during the experiment blood samples were collected.
weekly before feeding, via jugular venepuncture, into evacuated glass tubes (Beckman & Dickinson, Oxford, England). These samples were analysed for serum 3 hydroxy butyrate (3(OH)B), non-esterified fatty acids (NEFAs) and urea using the methods described by Hart et al. (1978). To determine the serum concentration of glucose, a separate blood sample was collected into iodoacetate-coated tubes (Teklab; Durham, England). These were analysed according to the method of Hart et al. (1978).

Statistical analyses

To compare the data from the pregnant ewes at the same stage of pregnancy, only the data from the six weeks before actual lambing were used. This was because the exact dates on which the pregnant ewes conceived were not known. Comparisons were made between the pregnant ewes that were home-bred and those that were bought in.

The results from the ewes allocated to the food choices, A/B and C/D, and the single foods, A, B and C, were treated as a randomised design and were analysed using GENSTAT 5 (Lawes Agricultural Trust, 1987). A multiple linear regression model was used to obtain the comparisons; it was necessary to analyse the data in this manner because the design was non-orthogonal. Feeding treatment and physiological state (pregnant and non-pregnant) were used as explanatory variables. The body condition scores of the ewes and the serum concentrations of NEFAs, 3(OH)B, urea and glucose were analysed using a multiple linear regression model with feeding treatment, physiological state and week of sampling/measurement used as explanatory variables. The results are presented as means for the feeding treatments; the two physiological states are presented separately. Standard errors are given with each mean.
RESULTS

Health

One of the non-pregnant ewes was removed from the trial because she was found to be pregnant on a later scanning. A pregnant ewe allocated to food C was removed because she was wrongly assumed to be non-pregnant. One of the pregnant ewes offered food A as a single food was diagnosed to be at risk of developing pregnancy toxaemia and so was removed from the trial six weeks before lambing. She produced triplets without incurring any further problems. A pregnant ewe offered food C alone died from hypocalcaemia five weeks before the majority of the pregnant ewes lambed.

The pregnant and non-pregnant ewes offered food B alone had low food intakes relative to other ewes of the same physiological state. When the serum concentrations of 3(OH)B of several of these ewes rose above 1.2 m moles/l they all were removed from the experiment because there were serious concerns about their welfare, as it was felt that they were liable to develop pregnancy toxaemia (Robinson, 1980). After removal from the study, the pregnant ewes that had formerly been offered B as a single food were given one kg of food A per day and hay ad libitum; they lambed without further problems. Four of the six ewes produced triplets, although they had been assessed to be carrying twins. The data collected from these ewes have been excluded from the analyses. The other ewes all lambed without major problems. The number of ewes allocated to each treatment that generated data are shown in Table 2:2. There was no effect of source of the non-pregnant ewes upon any of the traits that were measured.

Daily food intake and diet selection

The daily food intakes of the ewes and the proportions of the high-protein food selected together with the CP and ME concentrations of the selected diets of the ewes are given in
Table 2: Table 2:3. The pregnant ewes sustained intakes that were similar to the non-pregnant ewes until lambing was imminent, when the intakes of the pregnant ewes declined rapidly (see Figures 2:1a and 2:1b which show the mean daily food intake of the pregnant and non-pregnant ewes offered the food choice, C/D and the single food, C respectively). As a result, the daily food intake of the non-pregnant ewes, when averaged over the six-week experimental period, was greater (P<0.01) than that of the pregnant ewes. The daily food intakes of the ewes on the other feeding treatments followed similar patterns. When offered the food choice of low ME concentration (C/D), both pregnant and non-pregnant ewes ate more food (P<0.001) than ewes offered the higher ME concentration food choice (A/B). Similarly the ewes offered the low ME and high CP food, C, consumed more than those offered the high ME and high CP food, A. Those barren ewes who were offered D (low ME: low CP) also consumed more than similar ewes offered food A (2569 g/d and 2769 g/d for barren ewes offered A and D respectively). The large standard error that is associated with daily food intake of the non-pregnant ewes offered C is due to one ewe whose intake was temporarily depressed. The 150g hay offered daily was consumed by all ewes.

When offered the food pair with a high ME concentration (A/B), the pregnant ewes selected a significantly (P<0.001) greater proportion of the food of high CP content than the non-pregnant ewes. In contrast, pregnant and non-pregnant ewes, which were given access to the food choice of low energy density (C/D), chose very similar proportions of the food of high CP content (C). This interaction between feeding treatment and physiological state was significant. The dietary choices made by both the pregnant and non-pregnant ewes were consistent throughout the experimental period.
Table 2:3: The effects of feeding treatment group (AB & CD two-way choices; A & C single feeds) for twin-bearing and non-pregnant ewes on daily food intake, the proportion of high-protein food selected by the ewes on the two-way choices, intakes of metabolisable energy (ME) and crude protein (CP). Values in brackets are standard errors.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non pregnant</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diet</td>
</tr>
<tr>
<td>No of ewes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Daily food intake (g/day)</td>
<td>1936 (141)</td>
<td>2898 (121)</td>
<td>2237 (99)</td>
</tr>
<tr>
<td>Daily food intake excluding wk 7 (g/day)</td>
<td>2061 (156)</td>
<td>3057 (115)</td>
<td>2369 (94)</td>
</tr>
<tr>
<td>Proportion of A or C selected</td>
<td>0.57 (0.074)</td>
<td>0.70 (0.070)</td>
<td>-</td>
</tr>
<tr>
<td>CP intake (g/d)</td>
<td>341 (37.2)</td>
<td>395 (28.0)</td>
<td>526 (22.3)</td>
</tr>
<tr>
<td>ME intake (MJ/day)</td>
<td>18.9 (1.51)</td>
<td>20.9 (1.39)</td>
<td>22.6 (1.00)</td>
</tr>
</tbody>
</table>

Level of significance: ns = P>0.05, * = P<0.05; ** = P<0.01 and *** =P<0.001,
a number of residual degrees of freedom=22.
Figure 2:1: Mean food intake (g/d) of pregnant and non-pregnant ewes during the last 42 days to parturition.
Performance of the ewes.

There were no significant effects of feeding treatment upon total lamb birth weight (sum of the birth weights of the lambs born to each ewe), nor was there a significant effect of either feeding treatment or physiological state upon the live-weight gain of the ewes over the first or second three week periods. (Table 2:4). Over the experimental period the body condition scores of all the pregnant ewes were greater than or equal to 3.5. They did not alter significantly over the six weeks to parturition (Table 2:4). The condition scores of the non-pregnant ewes rose from 3.75 (se 0.125) to 4.00 (se 0.125) over the same period. The non-pregnant ewes had significantly higher condition scores (P<0.001) than pregnant ewes on the same feeding treatment.

Serum concentrations of NEFAs, 3(OH)B, glucose and urea.

There were no significant effects of dietary treatment on the serum concentrations of 3(OH)B, NEFAs or glucose; as a consequence these results have been pooled across dietary treatment, producing the two groups, pregnant and non-pregnant, that are represented in Figures 2:2a, 2:2b and 2:2c. The circulating NEFA concentrations of the pregnant ewes were consistently higher (P<0.001) than those of the non-pregnant ewes (Figure 2:2b), whereas the serum 3(OH)B concentrations of the pregnant and non-pregnant ewes were not significantly different (Figure 2:2a). The non-pregnant ewes had significantly (P<0.001) higher serum glucose concentrations than the pregnant ewes. The serum 3(OH)B and NEFA concentrations of the pregnant ewes rose over the last six weeks before lambing (Figures 2:2a and 2:2b); over the same interval the serum glucose concentrations of the pregnant ewes declined (Figure 2:2c). The serum 3(OH)B concentration of the non-pregnant ewes fluctuated markedly (Figure 2:2a). The serum concentrations of urea nitrogen (Urea-N) did not alter significantly over the 6-week period in either the pregnant or non-pregnant ewes. The results from the different sampling weeks have been pooled across physiological state; these are given in Table 2:4.
Table 2.5 shows the estimated production of MP for each food or food choice and also the calculated ratios of effective rumen degradable protein (eRDP; see AFRC, 1992): fME (fermentable ME; see AFRC, 1992) generated.
Table 2.4: The effects of dietary treatment group (AB & CD two-way choices; A & C single foods) for twin-bearing and non-pregnant ewes on combined lamb birth weight, increase in ewe live-weight over weeks 1 - 3 and weeks 3 - 6 of the treatment, mean body condition score and serum urea N concentration. Values in brackets are standard errors.

<table>
<thead>
<tr>
<th>No of ewes</th>
<th>Pregnant</th>
<th></th>
<th>Non-pregnant</th>
<th></th>
<th></th>
<th></th>
<th>Feeding State Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AB 10</td>
<td>CD 6</td>
<td>A 5</td>
<td>C 4</td>
<td>AB 5</td>
<td>CD 5</td>
<td>A 5</td>
</tr>
<tr>
<td>Total lamb birth weight (kg)</td>
<td>10.5 (0.66)</td>
<td>8.5 (0.85)</td>
<td>10.1 (0.93)</td>
<td>11.6 (1.04)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Live-weight increase over wks 1 -3 (kg)_b</td>
<td>6.4 (1.17)</td>
<td>5.6 (0.51)</td>
<td>6.1 (1.50)</td>
<td>5.9 (0.70)</td>
<td>4.4 (0.48)</td>
<td>4.5 (1.10)</td>
<td>5.47 (0.74)</td>
</tr>
<tr>
<td>Live weight increase over wks 3 -6 (kg)_b</td>
<td>5.8 (1.40)</td>
<td>7.7 (1.79)</td>
<td>8.1 (2.23)</td>
<td>6.2 (1.80)</td>
<td>6.4 (1.07)</td>
<td>7.1 (1.27)</td>
<td>7.27 (1.13)</td>
</tr>
<tr>
<td>Condition score wk1</td>
<td>3.50 (0.13)</td>
<td>4.00 (0.13)</td>
<td>3.75 (0.13)</td>
<td>3.88 (0.13)</td>
<td>3.50 (0.13)</td>
<td>3.63 (0.13)</td>
<td>4.00 (0.13)</td>
</tr>
<tr>
<td>Condition score wk 6</td>
<td>3.50 (0.13)</td>
<td>3.75 (0.13)</td>
<td>3.75 (0.13)</td>
<td>3.50 (0.13)</td>
<td>3.88 (0.13)</td>
<td>3.75 (0.25)</td>
<td>4.00 (0.13)</td>
</tr>
<tr>
<td>Mean serum Urea-N (mg/100ml)</td>
<td>11.2 (0.76)</td>
<td>11.4 (2.07)</td>
<td>14.0 (0.75)</td>
<td>13.1 (1.20)</td>
<td>9.2 (0.54)</td>
<td>13.6 (2.11)</td>
<td>17.7 (1.81)</td>
</tr>
</tbody>
</table>

Levels of significance: ns = P>0.05 and *** = P<0.001

_b Weeks 1-3 and 3-6 of treatment are 6-3 and 3-1 weeks before actual lambing date respectively for the pregnant ewes.
Figure 2.2 Mean prefeeding serum concentrations of 3(hydroxy) butyrate, non-esterified fatty acids and glucose during the last six weeks to parturition of pregnant and non-pregnant ewes (with all feeding treatments combined) (Bar refers to standard error).
Table 2.5: The effect of feeding treatment group (AB & CD two-way choices; A & C single feeds) upon twin-bearing and non-pregnant ewes on ratio of effective Rumen Degradable protein (eRDP) to Metabolisable energy (excluding fat; fME), estimated metabolisable protein (MP) produced & ratio of MP/ME in the diets selected. Values in brackets are the standard errors.

<table>
<thead>
<tr>
<th>No of ewes</th>
<th>Pregnant</th>
<th></th>
<th>Non pregnant</th>
<th></th>
<th></th>
<th></th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AB 10</td>
<td>CD 6</td>
<td>A(^a) 5</td>
<td>C(^a) 4</td>
<td>AB 5</td>
<td>CD 5</td>
<td>A(^a) 5</td>
</tr>
<tr>
<td>eRDP/ fME ratio produced (g/ MJ)</td>
<td>12.0 (0.70)</td>
<td>10.4 (0.50)</td>
<td>16.5 -</td>
<td>12.9 -</td>
<td>8.8 (1.40)</td>
<td>11.1 (0.40)</td>
<td>16.5 -</td>
</tr>
<tr>
<td>Estimated MP produced (g /day)</td>
<td>183 (16.9)</td>
<td>212 (15.3)</td>
<td>256 (11.3)</td>
<td>226 (9.3)</td>
<td>193 (16.2)</td>
<td>262 (23.9)</td>
<td>294 (15.2)</td>
</tr>
<tr>
<td>MP/ ME (g/ MJ)</td>
<td>9.7 (0.64)</td>
<td>10.0 (0.37)</td>
<td>11.3 -</td>
<td>11.3 -</td>
<td>8.8 (0.82)</td>
<td>10.4 (0.27)</td>
<td>11.3 -</td>
</tr>
</tbody>
</table>

Levels of significance: ns= P>0.05; *=P<0.05 and ***=P<0.001

\(^a\) The eRDP/ FME and MP/ ME ratio produced were the same for all ewes allocated to these treatment.
DISCUSSION

The majority of pregnant ewes in this experiment carried twin foetuses to term without problem. The serum concentration of 3(OH)B of the pregnant ewes rose over the last six weeks before lambing (Figure 2:2a); with the exception of the ewes offered food B alone, the concentration of 3(OH)B did not exceed 1.1 mmoles/l which was suggested by Russel (1984) to be critical. Over the same period serum NEFA concentrations rose (Figure 2:2b) for all pregnant ewes, but such increases may be a consequence of normal changes in the endocrine status of ewes in late pregnancy (Lindsay, 1973).

The pregnant ewes given food B alone had to be removed from the experiment because they showed symptoms of rumen acidosis. These ewes were unable to exercise any dietary choice other than to eat or not to eat. It is important to note that pregnant and non-pregnant ewes offered the choice A/B showed no symptoms of acidosis, nor was the B food avoided completely, which suggests that food A or its combination with food B had an ameliorating influence on digestive function in contrast to food B alone.

The mean daily food intakes of the pregnant ewes over the last six weeks of gestation were lower than those of the non-pregnant ewes. This difference was due to the sharp drop in food intake when lambing was imminent. If data collected during this period are excluded from the analysis, the food intakes of both these groups were not statistically different (Table 2:3). In this experiment the daily food intake of pregnant ewes declined at a later stage than in some previous experiments (Forbes, 1970; Foot & Russel, 1979). It is likely that the earlier decline of food intake that was found in those experiments can be attributed in part to the bulky nature of the foods which were used (Hunter & Siebert, 1986).

It appears that ewes offered the food choice (C/D), and those offered foods C or D alone, were able to compensate for the low ME concentration of the foods offered by eating significantly
more than ewes of the same physiological state which were offered the food choice of higher ME concentration (A/B). It has been shown by many groups (for example Owen et al. 1969) that sheep are able to compensate for the energy dilution of foods to a greater degree when the foods are ground and pelleted. As a result of these adjustments in food intake, feeding treatment had no significant effect upon the total birth-weight of lambs nor on the live-weight gain of the non-pregnant ewes.

The ME intakes of the pregnant ewes exceeded the ARC (1980) estimates of the requirements of twin-bearing ewes by about 20 % until the last ten days before parturition, and therefore would have been sufficient for some maternal live-weight gain over the last six weeks of gestation which was in addition to the growth and development of the foetus and associated maternal tissues. The ME intakes of the non-pregnant ewes exceeded the estimated maintenance requirement of non-pregnant ewes, housed in a similar manner (0.33 MJ/ W0.75, Heaney & Lodge, 1975) by a factor of 3.5. The non-pregnant ewes all gained a considerable amount of live-weight over the six-week period; there was no effect of the feeding treatment on live-weight gain. It is entirely possible that the non-pregnant ewes used this opportunity to increase their level of fatness and body weight toward a mature level of fatness (seen by Blaxter et al. 1982).

It is apparent that considerable control was exercised by all the pregnant ewes with respect to their intakes of ME. The pregnant ewes given a choice between two foods demonstrated a similar degree of control over CP intake (Table 2:3), a possibility that was not available to the ewes given one food alone. The estimated MP intakes of all pregnant ewes were in excess of the AFRC (1992) estimated requirements of twin-bearing ewes in late pregnancy. Serum urea-N concentrations were high and did not alter significantly as the experiment progressed; they largely reflect differences in the crude protein intakes of the ewes (Table 2:3). It is therefore concluded that the ME and protein intakes of all the pregnant ewes were at least adequate to meet requirements for both foetal and maternal growth and development.
If the diet selection of the ewes is seen as a set of rules whose precedence depends upon the choice of foods offered to the animals (Emmans, 1991), then the rule followed by the ewes offered the low ME concentration food choice (C and D) may have been different from that followed by the ewes given the high ME concentration food choice (A and B). When offered foods A and B as a choice, non-pregnant ewes given this food choice selected significantly less of the high CP food, A, than the pregnant ewes. In contrast both the non-pregnant and pregnant ewes given foods C and D as a choice selected high proportions of the high-CP food, C. This suggests that the physiological state of the ewes is an important influence upon food choice with a food pair of high energy density, but not when the food pair on offer was of lower energy density. An important common factor which could have led both the pregnant and non-pregnant ewes to select for a relatively high proportion of the high protein food, C, is the influence of the protein in the food on rumen function with consequences for ingestion of a food of lower energy density.

The supply of resources for microbial protein production may be of significance to the ruminant, both to supply MP (AFRC, 1992) and to facilitate digestion and intake. The ratio of eRDP: fME is a measure of the balance between the supply of energy from fermentation and protein to the rumen micro-organisms. When the rates of supply of energy-yielding and protein-yielding substrates are not in balance for the rumen micro-organisms, the consequence for the animal may be a reduced supply of potentially digestible microbial material, and perhaps also an accumulation in the rumen of the end-products of carbohydrate or crude protein digestion. An imbalance in the rates of degradation of feed carbohydrate and crude protein could lead to an accumulation of ammonia (Oldham et al. 1977).

Selection between food of widely differing eRDP/ fME ratios in order to minimise disruption to effective rumen function, or accumulation of end products of fermentation, may therefore be a strategy which is beneficial to ruminants, especially when food intake is likely to be constrained by the fermentative activities of rumen micro organisms. The calculated eRDP/
fME ratios in the diets chosen by the pregnant and non-pregnant ewes offered the choice C/D were very similar (10.4 se (0.500); 11.1 se (0.400), respectively). It seems unlikely that sheep would select for 'optimal microbial growth' per se because they would have no means of assessing this directly. Instead, diet selection could be affected by the consequences of imbalanced rumen conditions - which might include elevated ammonia concentrations, shifts in VFA concentration, acidity, osmolality (Engku Azahan & Forbes, 1992) or alternatively a deficit in the supply of MP to the body. We cannot from our results identify which, if any, of these may have been responsible for directly affecting diet selection. However the maintenance of a rumen environment which was optimal for microbial growth would have the consequence of allowing the animals to achieve the high rate of food intake necessary to meet its ME requirements. The eRDP/ fME ratio in the diet selected by the pregnant and non-pregnant ewes offered foods A and B as a choice were 12.0 (se 0.70) and 8.8 (se 1.40) respectively. In every case the ratio chosen was within the range identified by AFRC (1992). This coincidence, if fortuitous, is remarkable, especially as the differences between the individual foods in each pair in eRDP/ FME ratio were very considerable. The observation raises the intriguing possibility that these ewes were making selections which optimised rumen function and thereby facilitated achievement of high 'target' ME intakes.

However, it is a possibility that the non-pregnant ewes made their diet selections for reasons that were not strictly nutritional; for example on the basis of the organoleptic properties of the foods. Such an explanation excludes the possibility that sheep are capable of exhibiting nutritional wisdom (Groven & Chapman, 1988) and could not form the basis of a unique theory that also accounts for the diet selection of the C and D ewes. In the light of other evidence to the contrary (Engku Azahan & Forbes, 1992; Kyriaazakis & Oldham, 1993), we would reject this possibility.

The second objective of the experiment was to alter, by dietary means, the availability of non amino-acid glucogenic precursors (propionate). The intention of doing this was to test whether
pregnant ewes will alter their selections between foods of different protein content in order to remedy a deficit of glucogenic precursors from other sources (propionate). It was intended that the rumen fermentation of foods C and D would yield a lower molar proportion of propionate than that of A and B, and so it was hypothesised that pregnant ewes on the choice CD would select diets that would enable them to have a higher protein intake than those offered the choice A/B. The pregnant ewes offered the choice C/D did select a significantly higher proportion of the high CP food than those offered the choice A/B, although their absolute intakes of CP did not differ. It is possible that such diets were selected by the pregnant ewes in order to increase the availability of glucogenic amino acids, given the increased glucose demand imposed by the presence of foetuses (Lindsay, 1973). However it is unlikely that the non-pregnant ewes selected a similar proportion of food C in their diet selections for this reason. The observation discussed above, that selection may have been determined, for C/D especially, by rumen conditions provide an alternative hypothesis. Unfortunately it was not possible to monitor the ruminal molar proportions of propionate and acetate as intact sheep were used, therefore it is not known whether the fermentation of food C and D in the rumen did yield a lower proportion of propionate than foods A and B.

In summary it appears that the pregnant ewes were able to exhibit state-dependant selection of diets which enabled them to supply their own needs and the increasing requirements of the foetuses. It was not possible to determine whether the availability of non amino -acid glucogenic precursors prompted the ewes to alter their diet selection. When offered the high ME concentration food choice (A/B), the physiological state of the ewe and associated changes in protein demand are proposed as the main priorities guiding diet selection. Dietary constraints may have superseded the priorities of the physiological state when the ME content of the food choice was lower (C/D), and in this circumstance diet selection may well have been adjusted to optimise the rumen environment in terms of its ability to facilitate a high rate of ME intake.
Chapter Three

Diet selection in Sheep: the role of the rumen environment on the selection of a diet from two foods that differ in their energy density

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ABSTRACT

DIET SELECTION IN SHEEP: THE ROLE OF THE RUMEN ENVIRONMENT ON THE SELECTION OF A DIET FROM TWO FOODS THAT DIFFER IN THEIR ENERGY DENSITY

The effect of the energy density (ED) of foods offered as choice on the diet selection of sheep, and the relationship between the rumen environment and the diet selected from foods of different ED, were investigated in two experiments. In the first experiment two foods L and H and their mixture M (3:1 w/w) were formulated: All foods had similar calculated metabolisable protein: metabolisable energy (ME) ratios, but differed in ED (7.4, 8.1 and 10.1 MJ ME per kg fresh food for L, M and H respectively). The foods were offered ad libitum either singly or in paired choices (L/M, L/H and M/H) (n=6 per treatment) to growing sheep. Although the rate of live-weight (LWT) gain on food H was higher than foods L or M, and the daily rate of food intake lower, the sheep given food choices L/H and M/H did not consume only food H. Instead they selected a mixture of both foods offered such that the total amount of H consumed per kg fresh food was similar on choices L/H and M/H. The rate of LWT gain of sheep on choices L/H and M/H was not different from that achieved on food H alone. In the second experiment the choice L/H was offered to rumen-fistulated sheep (10 months of age, mean LWT 57.5 kg) in an 8x8 Latin square, with 7 periods. Treatments were infusions into the rumen (total volume 1 l) over 4 hours on days 4 - 7 of each period of Acid (HCl; Acid 1: 400, Acid 2: 300 & Acid 3: 200 mMol/l), Alkali (NaOH; Alk 1: 316, Alk 2: 212 & Alk 3: 109 mMol/l) and Control (NaCl; Con 1: 315 & Con 2: 209 mMol/l). Infusate osmolalities (mOs/kg) were 795 (Acid 1), 585 (Acid 2, Alk 1 & Con 1), 390 (Acid 3, Alk 2 & Con 2) and 200 (Alk 3). Infusion treatment significantly affected the diet selection of the sheep (P<0.05), according to the osmolality of infusate but not rumen pH. During infusions intake of food H tended to decline with increasing treatment osmolality, whereas intake of L remained constant.
The effects on diet selection and food intake were of a short duration with no carry-over effects. These results show that sheep given a choice between two foods of different ED select a substantial quantity of the low ED food; this diet selection is affected by short-term manipulations of their rumen environment in a manner that is consistent with the maintenance of effective rumen conditions.

INTRODUCTION

It is a precept of diet selection that animals aim to optimise the advantages to be gained by eating a food, such as rapid growth and production of viable offspring, whilst minimising any disadvantages which may be incurred (Emmans, 1991). This assumption has been the underlying hypothesis of most research into diet selection and it is implied in optimal foraging theory (Krebs & McCleery, 1984). Research into the diet selection of animals in a controlled environment has concentrated upon their ability to select, from a choice of foods differing only in the concentration of one nutrient (such as protein), a diet which meets their requirements for that nutrient (chickens: Shariadmadari & Forbes (1993); pigs: Kyriazakis et al. (1990); sheep: Cropper (1987); Kyriazakis & Oldham (1993)). The diets selected by the animals in those experiments have enabled them to grow rapidly, and yet avoid an excessive intake of the nutrient in question, since this could present a metabolic burden (for example protein: Kyriazakis & Oldham (1993)).

It is predicted from optimal foraging theory (Krebs & McCleery, 1984) that the diet selected by ruminants, offered a choice between foods of different digestibility would consist almost exclusively of the food which enables them to maximise their rate of intake (Westoby, 1974; Kenney & Black, 1984). However, evidence from the grazing experiments of Newman et al. (1992) and Parsons et al. (1994) do not support this hypothesis. In their experiments it has
been shown that the sheep selected a mixture of the foods available to them (monocultures of grass and clover), although they had the opportunity to select a diet composed of clover alone which, from conventional expectations would have allowed them to maximise their rate of food intake (Kenney & Black, 1984). In addition, Cropper (1987) found that (in a controlled environment), sheep which were offered a free choice of a pair of foods differing in digestibility, did not completely avoid the less digestible food; instead they choose to eat a mixture of both foods. However the food choices offered by Cropper (1987) did not provide as wide a range of 'bulk densities' as had been intended, and also the number of animals allocated to each treatment was small; it is felt that this may have had a bearing upon the results obtained (Cropper, 1987). The objective of the first experiment reported here was, therefore, to give further consideration to the effects of energy density on the diet selection made by sheep.

Both Cropper (1987) and Parsons et al. (1994) have suggested that ruminants appear to select from two foods that differ in nutrient density or digestibility a diet that enables their rumens to remain in a fit and adaptive state. This strategy would require certain aspects of the rumen environment to remain at optimal levels, or at least within an acceptable range of conditions. Microbial activity within the rumen is greatly affected by changes in the rumen environment (Russel & Strobel, 1993). This is of significance to the sheep as the supply of energy and protein to the small intestine depends principally upon the activity of these micro organisms. Thus, if a strategy of maintaining optimal rumen conditions were to influence the diet selection of ruminants, this would assist the animal in achieving its goal of meeting its requirements for energy and nutrients.

One can hypothesise that the rumen conditions that may have a significant effect upon the diet selection of ruminants would be those which are related to the consequences of fermentation of rapidly fermentable materials (such as increased acidity and increased osmolality) and the
hydrolysis of rapidly degradable protein (high concentrations of ammonia). We have some evidence for the latter; since one of the criteria that influences the diet selection of sheep appears to be the 'desire' to minimise an excessive intake of rapidly degradable protein (Kyriazakis & Oldham, 1993; Cooper et al. 1994). It is known that the intake of a single food is affected by both rumen pH (Bhattacharya & Warner, 1967) and osmolality (Carter & Grovum, 1990a). The objective of the second experiment reported here was to investigate the effects of these aspects of the rumen environment (pH and osmolality) on the diet selection of sheep given a choice between two foods that differ in their energy density.

**MATERIALS & METHODS**

**Experiment 1**

*Animals & Housing*

Forty-two Suffolk x Greyface entire male lambs were used. They weighed 21.2 (sd 5.53) kg live-weight (LWT) when they were weaned at approximately 8 weeks of age. The lambs were drawn from sets of twins from which one was taken. The lambs were given a creep feed (158 g CP per kg fresh food; 10.1 MJ metabolisable energy (ME) per kg) at a rate of 3.5 % of Lwt/day. It was estimated that this feeding level, which was very close to their *ad libitum* intake, would ensure that all the sheep reached the desired experimental live-weight with a similar gut fill. They were kept in individual pens (1.29 x 1.53 m) which contained one water bucket and one or two food troughs (according to the experimental treatment). The shed in which they were housed has been described previously by Kyriazakis & Oldham (1993). Natural lighting and ventilation was used throughout the experiment (May-September 1992).
**Foods**

Two foods, L and H, were formulated and made into pellets (Table 3:1), they differed mainly in their energy density (Low (L) and High (H)). Both foods had estimated Metabolisable Protein: Metabolisable Energy ratios (MP:ME) that were made as similar as possible within the constraint of ensuring that the effective rumen degradable protein (eRDP): fermentable ME (fME) ratio was kept constant. The MP content of the foods was estimated using the system proposed by the Agricultural and Food Research Council (1992) and the method of Thomas et al. (1988) was used to calculate the ME content of the foods. Food L was formulated to have inadequate concentrations of ME and MP to support potential growth when offered *ad libitum* to sheep (AFRC, 1992). Both foods were non-limiting in minerals and vitamins but in food H the concentration of macro-minerals was higher to maintain suitable ratios to metabolisable energy (Agricultural Research Council, 1980). A mixture of foods L and H was also made, M (3/4 L: 1/4 H).

**Design**

Upon reaching 30 kg Lwt each sheep was allocated to one of three groups: initial slaughter (n=6), free and continuous access to a single food (L, M and H; n=6 per treatment; single fed) or a free and continuous access to a choice between two foods (food pairs L with M (L/M), L with H (L/H) and M with H (M/H) n=6 per treatment; choice fed). The sheep were allocated randomly to the treatments after taking into account age at the starting live-weight. The experiment ended when the sheep reached 50 kg LWT, (for all the single fed sheep and those offered choices between foods L and M or foods M and H). The sheep allocated to the L/H choice continued to be offered these two foods until they reached 60 kg Lwt; this was to consider whether their food intake and diet selection changed with time, and more specifically with natural day-light.
Table 3.1: Ingredients and chemical composition of the experimental foods (Experiments 1 and 2).

<table>
<thead>
<tr>
<th>Ingredients (g/kg)</th>
<th>Foods</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>M</td>
<td>H</td>
</tr>
<tr>
<td>Oatfeed</td>
<td>642.6</td>
<td>557.0</td>
<td>300.0</td>
</tr>
<tr>
<td>Barley</td>
<td>-</td>
<td>21.7</td>
<td>86.6</td>
</tr>
<tr>
<td>Molassed beet pulp</td>
<td>102.9</td>
<td>153.9</td>
<td>306.8</td>
</tr>
<tr>
<td>High protein soya</td>
<td>180.2</td>
<td>194.2</td>
<td>236.2</td>
</tr>
<tr>
<td>CMS 20</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Salt</td>
<td>4.9</td>
<td>4.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Dicalphos</td>
<td>7.3</td>
<td>6.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Limestone flour</td>
<td>8.1</td>
<td>8.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Calcined magnesite</td>
<td>1.9</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Mineral and vitamin mix</td>
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<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1000.0</strong></td>
<td><strong>1000.0</strong></td>
<td><strong>1000.0</strong></td>
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</table>

Chemical analyses (g/kg fresh matter unless otherwise stated)

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>M</th>
<th>H</th>
</tr>
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<tbody>
<tr>
<td>Dry matter</td>
<td>891.0</td>
<td>888.0</td>
<td>875.0</td>
</tr>
<tr>
<td>Metabolisable energy (ME; MJ/kg)</td>
<td>7.4</td>
<td>8.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Fermentable ME (fME;MJ/kg)</td>
<td>6.6</td>
<td>7.4</td>
<td>9.9</td>
</tr>
<tr>
<td>Crude protein</td>
<td>124.0</td>
<td>138.0</td>
<td>178.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>22.4</td>
<td>23.6</td>
<td>26.3</td>
</tr>
<tr>
<td>Effective rumen</td>
<td>78.7</td>
<td>87.8</td>
<td>114.9</td>
</tr>
<tr>
<td>degradable protein (eRDP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eRDP/fME</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>440.0</td>
<td>402.0</td>
<td>286.0</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>214.0</td>
<td>192.0</td>
<td>125.0</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>170.1</td>
<td>157.2</td>
<td>107.6</td>
</tr>
<tr>
<td>Ash</td>
<td>66.0</td>
<td>67.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.2</td>
<td>8.9</td>
<td>10.9</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.5</td>
<td>3.7</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Estimated production (g/kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial protein</td>
<td>41.9</td>
<td>52.7</td>
<td>63.5</td>
</tr>
<tr>
<td>Metabolisable protein (MP)</td>
<td>73.9</td>
<td>83.4</td>
<td>111.8</td>
</tr>
<tr>
<td>Estimated ME: MP (g/MJ)</td>
<td>10.0</td>
<td>10.2</td>
<td>11.0</td>
</tr>
</tbody>
</table>

a Mineral and vitamin mix used was Scotmin ewe/lamb (Scotmin Nutrition Ltd, Ayr, Scotland).
b ME calculated using the following equation (ME=0.14 (Neutral cellulase gaminase digestibility) + 0.25 (Acid Hydrolysed ether extract); Thomas et al. (1988)).
c Values calculated using the Metabolisable protein system (AFRC, 1992), assuming a rumen outflow rate of 0.05 h⁻¹ and standard values for degradability coefficients.
Management & Slaughter Procedure

The sheep to be given a choice between two foods were given a training period of 10 days, in which the foods were offered alone on alternate days; the regime used was a modification of that which was developed by Kyriazakis & Oldham (1993). The quantity of food offered to all sheep was increased from 3.5 % Lwt to ad libitum over this period. The sheep were weighed weekly during the afternoon up to 47 kg Lwt (or 57 kg Lwt; L/H choice) and then daily (during the morning, before feeding) until they reached 50 kg Lwt (or 60 kg Lwt; L/H choice). They were offered fresh food twice daily (morning and afternoon) to minimise spillage; food refusals were collected daily, weighed and then discarded.

Each of the single-fed sheep was slaughtered on the morning that it reached 50 kg Lwt. Prior to slaughter the sheep was sheared closely, the wool was collected, cleared of any obvious dirt and then weighed to give the greasy fleece weight. The sheep were killed by an intravenous injection of Pentobarbital sodium (Euthatal- RMB). The dead weight of each sheep was recorded before the gastrointestinal tract was removed and weighed. The reticulo-rumen, omasum, and abomasum were removed together from the rest of the tract, this set of organs has subsequently been described as the 'stomachs'. Any omental fat was removed from the 'stomachs' before they were weighed, stripped of their contents and then reweighed. The small and large intestines were stripped of their contents and weighed empty, after the mesenteric fat had been removed. The weights of the contents of the 'stomachs' and the rest of the gastrointestinal tract were then calculated by difference.

Experiment 2

Animals & Foods

Eight Texel x Scottish Blackface female sheep (aged 10 months), weighing 57.5 kg (sd 6.92) LWT, were used. Each animal was fitted with a rumen cannula under surgical anaesthesia (O₂/ Halothene general anaesthesia), three months before the start of the experiment. During
the experiment the sheep were kept in metabolism crates, placed in a naturally ventilated room; the sheep were given a minimum of 12 hours of light. The sheep had been given prior experience of the cages, the procedures to be used and the experimental routine during a 23 d. pilot study.

The sheep were introduced to the experimental foods in an alternating pattern three weeks before they were fitted with a rumen cannula. They were given free and continuous access to the foods at all times until the end of the experiment. The food choice offered consisted of the high energy density food, H, and the low energy density food, L, used in Experiment 1. The foods were given in two identical food troughs; water was available at all times, but its intake was not monitored. Fresh food and water was given each day.

**Design**

The experiment was an 8 x 8 Latin square design (eight treatments, each period was one week in length). There was a 1 week interval, dividing the experiment into two 4 week sections, to allow the animals a period of rest so that they were not held in metabolism crates for more than 4 weeks, in accordance with Home Office regulations. The treatments were rumen infusions of one litre and were administered to the sheep over a four-hour period (1000 - 1400 h) on four consecutive days (1 - 4) of each of the eight weeks of the experiment. There was an interval of three days between successive treatments (days 5-7) to avoid carry over effects. The treatments were three concentrations of HCl (Acid 1-3, treatments), three of NaOH (Alkali 1-3, treatments) and two concentrations of NaCl (Control 1 and 2, treatments). HCl was chosen for the Acid treatments in preference to an organic acid as it would have an effect upon pH without acting as a source of energy. NaCl was chosen as the Control as it would not cause rumen pH to be altered and its osmotic effects at specified concentrations could be easily predicted (Schiller et al. 1988). The molarity and osmolality of the solutions is given in Table 3.2. An in vitro study of the buffering capacity of rumen liquor taken from the sheep
during the pilot study indicated that infusions of the Acid treatment of highest concentration (Acid 1) or the Alkali of highest concentration (Alkali 1) would cause equal but opposite changes in rumen liquor pH. Acid 2, Alkali 1 and Control 2 had the same high osmolality, whilst Acid 3, Alkali 2 and Control 2 were of a lower osmolality.

Table 3:2: The concentration and osmolalities of the solutions in Experiment 2 infused into sheep given access to two foods that differed in energy density.

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Treatment</th>
<th>Concentration (mM)</th>
<th>Osmolality (mOs/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>Acid 1</td>
<td>400</td>
<td>795</td>
</tr>
<tr>
<td></td>
<td>Acid 2</td>
<td>300</td>
<td>585</td>
</tr>
<tr>
<td></td>
<td>Acid 3</td>
<td>200</td>
<td>390</td>
</tr>
<tr>
<td>NaOH</td>
<td>Alkali 1</td>
<td>316</td>
<td>585</td>
</tr>
<tr>
<td></td>
<td>Alkali 2</td>
<td>212</td>
<td>390</td>
</tr>
<tr>
<td></td>
<td>Alkali 3</td>
<td>109</td>
<td>200</td>
</tr>
<tr>
<td>NaCl</td>
<td>Control 1</td>
<td>315</td>
<td>585</td>
</tr>
<tr>
<td></td>
<td>Control 2</td>
<td>209</td>
<td>390</td>
</tr>
</tbody>
</table>

Management & Measurement

Food refusals were removed at 0800 hrs each day, weighed and discarded. Food consumption was recorded at 1000 and 1600 hrs each day. On days 1 - 4 food intake was measured every two hours between 0800 and 1600 hrs by removing the troughs, weighing them and returning them to the pens. The live weight of the ewes was measured on day 7 of each week before feeding. The rumen infusates were administered via a piece of semi-rigid PVC tubing (internal diameter (i.d.) 3 mm) inserted through a rumen cannula bung. On the fourth day of rumen infusion in each block (day 4), volumes of c 80 ml rumen liquor were withdrawn at 0800, 1000, 1200, 1400 and 1600 hours. To take rumen samples, a stiff piece of tubing
(polypropylene; i.d. 9 mm) was inserted through the rumen cannula bung, this was adjacent to the infusion tubing but did not touch it. The rumen liquor samples were withdrawn using a hand held pump, they were collected into glass containers. Efforts were made to collect the samples from the same parts of the reticulo-rumen (different sampling sites) at each sampling time. Small rubber plugs were used to seal the rumen cannula bung when infusion and sampling procedures were not being conducted. In addition, on day 2 blood samples were collected into evacuated heparinised tubes by jugular venepuncture, at 0900, 1200 and 1500 hrs.

**Sample processing and analysis**

The pH of each rumen liquor sample was measured immediately after sampling, using a glass electrode, the rumen liquor was then strained through double thickness muslin. Strained rumen liquor samples were stored at -20°C and were subsequently analysed to determine ruminal concentrations of volatile fatty acids (VFAs) and ammonia (NH₃N). Separate samples for VFA and NH₃N analyses were treated with 250 µl saturated mercuric chloride or 3 drops of concentrated sulphuric acid respectively before freezing. The rumen fluid was analysed to determine the concentrations of individual VFAs using a gas - liquid chromatograph (GLC, Model 304, Pye Unicam Ltd; Cambridge) and for NH₃-N on an Autoanalyser (Kjeltec 1030 Autoanalyser). Repeated attempts were made to measure the osmolality of the rumen liquor samples that had been filtered through 0.45µm microbial filters (Whatman Ltd, Maidstone, England) but these were not successful as the liquor was too viscous for reliable measurements to be taken.

Blood samples were centrifuged (1500 g; 20 minutes) and 1 ml aliquots of plasma were mixed with 6 µl Lithium Chloride solution (concentration 750 mM) and stored at -20°C. These samples were analysed for Na, Cl and total CO₂ (tCO₂) with commercially available kits, using a micro-centrifugal auto-analyser (Monarch 2000, Instrument Laboratory, Warrington).
Statistical Analyses

**Experiment 1**

All statistical analyses were performed using GENSTAT version 5.3 (Lawes Agricultural Trust, 1988). Treatment effects for the single and choice-fed sheep in Experiment 1 were analysed by ANOVA. Comparisons between treatments were made using orthogonal contrasts. The rate of liveweight gain and food conversion efficiency were analysed by including data from the 10 day training period in the first instance (Whole period) and then by excluding data from the training period (Experimental period). The rate of liveweight gain over the Whole period was calculated as the quotient of liveweight gained over this period and the time taken. For each animal a simple linear regression was used to calculate the rate of liveweight gain over the Experimental period. The rate of empty body weight gain (single fed sheep only; liveweight excluding the weight of the contents of the gastrointestinal tract) was calculated over the Whole period.

**Experiment 2**

Data from this experiment were analysed according to an 8 x 8 Latin square design. Daily food intake and the proportion of H selected were analysed with day and treatment as factors. Secondly the effects of day and treatment on the food intake and diet selection during the four days of the infusion were tested. The data were divided into three time intervals (0800 - 1000 h), (1000 - 1600 h) and (1600 - 0800 h) which were analysed separately. The food intakes and diet selections during these intervals were then analysed using day and osmolality within treatment (treatment/ osmolality) as factors. The pH, NH$_3$N and VFA data (collected on day 4 of each week), and the plasma concentrations of sodium, chloride and total carbon dioxide were analysed in two ways. Firstly, data from the five sampling times (three times for blood sampling) were analysed separately; data from the 0800 hr sampling (for pH, NH$_3$N and VFA) and 0900 hr sampling (for plasma concentrations) were used as the respective covariates. Subsequently, mean data from the infusion period were treated in a similar manner.
to the food intake and diet selection data, using treatment as a factor. To ensure that the data
distribution met the assumption of normality, the following variates were transformed: total
VFAs ($X^2$), molar proportion of isobutyrate ($\log_{10}$), and isovalerate (sqrt); the terms within
the parentheses indicate the transformation used. The diet selection data from both
Experiments 1 and 2 were not transformed by any means, since in both cases they met the
criteria for a normal distribution.

RESULTS

Experiment 1

Single fed sheep

The rates of daily food intake, live-weight gain, food conversion efficiency, empty body
weight gain and daily ME intake of the sheep given free and continuous access to a single food
are given in Table 3:3. Two values are quoted for both rate of live-weight gain and food
conversion efficiency. The former includes the 10 day training period (Whole period) and the
latter excludes it (Experimental period). Both food intake and food conversion efficiency
increased significantly ($P<0.001$) as the energy density of the food decreased. This effect of
energy density upon food conversion efficiency was apparent over the Whole period and the
Experimental period. The sheep fed on H alone had significantly higher rates of liveweight
gain ($P<0.05$) over the Experimental period, however this difference was not present when the
Whole period was considered. The rate of empty body weight gain (liveweight excluding gut
fill) on food H was significantly higher than on foods L or M alone.

The weights of the contents of the rumen, reticulum, omasum and abomasum ('stomachs') and
the remaining sections of the gastro intestinal tract are given in Table 3:3. The 'stomachs'
contents of the sheep on H weighed significantly less than the sheep on foods L or M. There
was no effect of energy density on either the contents of the remaining sections of the gastro
intestinal tract or the 'cleaned' tissue weights of the entire gastro-intestinal tract or the 'stomachs' which were: 2030, 1949, 2050 (sed 152.2) g for foods L, M and H respectively.
Table 3.3: The performance of sheep in Experiment 1 that were given access to foods with different calculated energy densities (ED; MJ ME/ kg fresh food) but similar protein: energy ratios, from 30-50 kg lwt.

<table>
<thead>
<tr>
<th>Food</th>
<th>Calculated ED</th>
<th>L</th>
<th>M</th>
<th>H</th>
<th>SED (14 df.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>2802</td>
<td>2769</td>
<td>2107</td>
<td>110.0</td>
<td></td>
</tr>
<tr>
<td>Live-weight gain (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole period</td>
<td></td>
<td></td>
<td>463</td>
<td>471</td>
<td>512</td>
</tr>
<tr>
<td>Experimental period</td>
<td></td>
<td>421</td>
<td>443</td>
<td>503</td>
<td>31.1</td>
</tr>
<tr>
<td>Food conversion efficiency</td>
<td>(g gain/ g food)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole period</td>
<td></td>
<td></td>
<td>0.187</td>
<td>0.193</td>
<td>0.268</td>
</tr>
<tr>
<td>Experimental period</td>
<td></td>
<td>0.151</td>
<td>0.160</td>
<td>0.244</td>
<td>0.0122</td>
</tr>
<tr>
<td>EB wt gain (g/d)</td>
<td></td>
<td></td>
<td>341</td>
<td>361</td>
<td>423</td>
</tr>
<tr>
<td>ME intake (MJ/d)</td>
<td></td>
<td>22.9</td>
<td>24.9</td>
<td>24.2</td>
<td>0.98</td>
</tr>
<tr>
<td>'Stomachs' contents (g)</td>
<td></td>
<td>7230</td>
<td>6197</td>
<td>5306</td>
<td>727.6</td>
</tr>
<tr>
<td>Remaining gastro intestinal tract contents (g)</td>
<td></td>
<td>5345</td>
<td>4852</td>
<td>3392</td>
<td>795.7</td>
</tr>
</tbody>
</table>

Statistical significance of effects of:

<table>
<thead>
<tr>
<th>Food</th>
<th>Orthogonal contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td>H vs others</td>
</tr>
<tr>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>NS</td>
<td>*</td>
</tr>
</tbody>
</table>

SED, standard error of difference; NS, not significant; ME, metabolisable energy; EB, empty body.

*, P< 0.05, ***, P< 0.001. L and H are low- and high energy density foods; M is a combination of L and H; for details of foods see Table 1.

Calculated by regression; Over live weight range 30-50 kg Lwt.
Choice-fed sheep

The performance and diet selection of the sheep given access to two foods as one of three choices during the Experimental period (which excludes the training period) are given in Table 3:4. The sheep showed a preference for the food of high energy density in each food choice offered: 830, 680 and 590 (sed 83) g/kg total food intake (TFI) for choices, L/M, L/H and M/H respectively. Since food M was a mixture of foods L and H (3/4 L: 1/4 H), it is possible to express the diet selections of all the choice-fed sheep as a selection between foods L and H. The proportion of H selected was not significantly different when the other food offered was either L or M (680 vs 695 g H/ kg TFI, sed 54.2). The average proportion of the higher density food selected by the sheep on food choices L/M, L/H and M/H is plotted against time in Figure 3:1; no systematic changes in the pattern of diet selection of the L/H sheep were observed. The diet selections of the L/M choice-fed sheep showed less daily variation than the choices made by the sheep offered the food choices L/H or M/H. The L/H choice-fed sheep continued to be offered this choice as they grew from 50-60 kg liveweight; the mean length of this period was 29 days (sem 4.5). The proportion of H selected by the L/H choice-fed sheep as they grew from 50-60 kg liveweight was 663 (sem 83.3) g H/ kg TFI; this was not significantly different from the proportion of H selected by the same animals over the lower liveweight range (30-50 kg).

There was no effect of food choice upon either the rate of live weight gain or food conversion efficiency. Over the experimental period the choice-fed sheep had higher rates of Lwt gain (P<0.05) than the single-fed sheep on L or M alone and their performance was not significantly different from that of the sheep on H alone.
Figure 3:1 Mean proportion of food with the higher ED selected in Experiment 1.
Table 3.4: The diet selection and performance of the lambs given access to two foods with different energy densities (MJ ME/ kg fresh food), but similar protein to energy ratios, from 30-50 kg Lwt.

<table>
<thead>
<tr>
<th>Food pair</th>
<th>L/M</th>
<th>L/H</th>
<th>M/H</th>
<th>SED (df 14)</th>
<th>Statistical significance of effects of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Choice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L/M vs L/H vs others</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L/H vs M/H</td>
</tr>
<tr>
<td>Food intake (TFI; g/d)</td>
<td>2654</td>
<td>2483</td>
<td>2326</td>
<td>89.1</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Proportion of food of food of higher energy density selected (g M or H/kg TFI)</td>
<td>834</td>
<td>680</td>
<td>592</td>
<td>81.1</td>
<td>NS</td>
</tr>
<tr>
<td>Proportion of food H selected (g/ kg TFI)</td>
<td>208</td>
<td>680</td>
<td>695</td>
<td>54.2</td>
<td>***</td>
</tr>
<tr>
<td>Live weight gain (g/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole period</td>
<td>491</td>
<td>494</td>
<td>482</td>
<td>26.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Experimental period</td>
<td>444</td>
<td>451</td>
<td>429</td>
<td>38.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Food conversion efficiency (g gain/ g food)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole period</td>
<td>0.209</td>
<td>0.226</td>
<td>0.224</td>
<td>0.0139</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Experimental period</td>
<td>0.163</td>
<td>0.181</td>
<td>0.187</td>
<td>0.0152</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>ME intake (MJ/d)</td>
<td>23.6</td>
<td>25.9</td>
<td>24.4</td>
<td>0.90</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

SED, standard error of difference; NS, not significant; ME, metabolisable energy. * , P<0.05; ** , P<0.01; *** , P<0.001; | L and H are low - and high energy density foods; M is a combination of L and H (3/4 L: 1/4 H) and therefore the diet selection of all sheep could be expressed as a selection between food L and H; for details of foods see Table 1. || calculated by regression; || over liveweight range 30 - 50 kg Lwt; na non applicable.
Experiment 2

Rumen pH

There was a significant effect of sheep and week on rumen pH (and all other measurements analysed). There was a significant effect of treatment at all sampling times during the 4-hour period of the infusion. The changes of rumen pH with time (0800 - 1600 h; day 4) are presented in Figure 3:2a (Acid treatments) and Figure 3:2b (Alkali treatments). The Control treatments were not significantly different from each other and so these two treatments have been combined for the purpose of presentation. The effect of treatment on rumen pH was still present two hours after the end of the infusion. Data from this interval (1400 - 1600 h) has been included in the analyses of all other measurements as a consequence. Rumen pH on Acid 1, Acid 2 and Alkali 1 treatments was altered significantly when compared with the Control treatments (Figures 3:2a and 3:2b) over the interval 1200 - 1600 h. The mean change in pH over the infusion period (1000 - 1400 h) on these treatments was 0.42 pH units. At the end of the infusion period rumen pH on these treatments began to return to levels comparable with the Controls.

Food intake

The food intakes over specific time intervals on the days when infusions were administered (Days 1 - 4) are shown in Table 3:5. There were clear tendencies for the treatment administered (0.1<p<0.05) to have an effect upon food intake during the four hours of the infusion and the two hours after they had ended (1000 - 1600 h). Food intake was depressed on the treatments of highest concentration within each treatment type, but this was not statistically significant. Food intake decreased significantly (P<0.05) with increasing treatment osmolality. There were no differences in the response to treatment osmolality due to treatment type, since there were no interactions between treatment type and osmolality.
The osmolality of the treatment which had been administered (1000 - 1400 h) had a significant
effect upon food intake during the interval (1600 - 0800 h); it is presumed that this was due to
the treatment Alkali 3 being particularly high relative to all the other treatments. Intake on day
2 (1564 (sem 56.9) g/d) was significantly depressed (P<0.05) relative to the other days when
infusions were administered; day 2 was the blood sampling day. There were no persisting
effects of the infusion treatments upon food intake on the days when no rumen infusates were
administered.

*Diet selection*

The diet selection results are presented as g food H/ kg TFI consumed during each time
interval considered (Table 3:6). There was a significant effect of treatment upon the
proportion of H selected (P<0.05) during the interval (1000 -1600h), which included the
infusion period. The proportion of H selected during the interval 1600 - 0800 h was not
affected by treatment, and neither was proportion of H selected on the non-infusion days
affected by treatment administered.

There was a clear tendency for proportion of H during the time interval (1000-1600) to be
depressed at high treatment osmolalities (0.1<p<0.05), this was irrespective of treatment type.
The treatment administered had a significant effect upon the intake of H, being 176, 174, 238,
168, 182, 273, 173 and 247 g/ 4 hours (sed 33) for Acid 1, Acid 2, Acid 3, Alkali 1, Alkali 2,
Alkali 3, Control 1 and Control 2 respectively. Intake of L during this time interval was not
affected by treatment, the mean being 180 (sem 8.9) g/ 4 hours. The osmolality of the
treatment tended to affect the intake of H during the interval (1000 - 1600 h), but it had no
effect on the intake of L, this is shown in Figure 3:3. Treatment osmolality had no effect on
the proportion of H selected outside this time interval.
3:2a Acid treatments

3:2b Alkali treatments

Figure 3.2 Mean pH of rumen liquor (0800-1600 h) of rumen fistulate sheep (Experiment 2) that were given 1l rumen infusions over a 4 hour period (1000-1400 h) (see Table 3:2) and offered a choice between foods L and H (for details see Table 3:1).
Table 3.5: The food intakes of the sheep in Experiment 2 that were given access to foods with different energy densities during the days when rumen infusate treatments were administered (days 1-4). For details of the treatments Acid 1 to 3, Alkali 1 to 3 and Control 1 and 2 see Table 3.2.

<table>
<thead>
<tr>
<th>Time Period (h.)</th>
<th>Acid 1</th>
<th>Acid 2</th>
<th>Acid 3</th>
<th>Alkali 1</th>
<th>Alkali 2</th>
<th>Alkali 3</th>
<th>Control 1</th>
<th>Control 2</th>
<th>SED (32 df.)</th>
<th>Infusate</th>
<th>Osmolality</th>
<th>Interaction</th>
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<tr>
<td>0800-1000</td>
<td>237</td>
<td>221</td>
<td>266</td>
<td>215</td>
<td>229</td>
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<td>189</td>
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<td>NS</td>
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<td>1000-1600</td>
<td>345</td>
<td>342</td>
<td>421</td>
<td>332</td>
<td>390</td>
<td>458</td>
<td>374</td>
<td>409</td>
<td>46.8</td>
<td>0.1&lt;p&lt;0.05</td>
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</tr>
<tr>
<td>1600-0800</td>
<td>929</td>
<td>924</td>
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<td>1234</td>
<td>1002</td>
<td>980</td>
<td>76.3</td>
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</tbody>
</table>

SED, standard error of difference; NS, not significant; *, P<0.05
Table 3.6: The diet selections made by the sheep in Experiment 2 that were given access to foods with different energy densities (MJ ME/ kg fresh food), during the days when rumen infusate treatments were administered (days 1-4): the data are presented as the proportion of food with high energy density (food H; g per kg total food intake) during the interval considered. For details of the treatments Acid 1 to 3, Alkali 1 to 3 and Control 1 and 2 see Table 3.2.

<table>
<thead>
<tr>
<th>Time Period (h)</th>
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<td></td>
<td>49.7</td>
<td>*</td>
<td>0.1&lt;p&lt;0.05</td>
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<tr>
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</table>

SED, standard error of the difference; NS, not significant; *, P<0.05
Figure 3:3 The mean food intake (1000-1600h) of rumen fistulate sheep that were offered foods L and H together (for details see Table 3:1); and which received rumen infusions of different osmolality (for details of infusates see Table 3:2).
Ruminal concentration of total VFAs, molar proportion of individual VFAs, ruminal concentration of NH$_3$ N, and plasma concentrations of Na, Cl, and CO$_2$.

The treatments administered had a significant effect (P<0.05) upon the total concentration of VFAs (tVFAs) at all sampling times of the four hours of the infusion period; the mean concentrations during the infusion period were: 108, 112, 125, 122, 138, 137, 120 and 128 (sed 7.8) mM tVFAs for Acid 1, Acid 2, Acid 3, Alkali 1, Alkali 2, Alkali 3, Control 1 and Control 2 respectively. There was no significant effect of treatment on the molar proportions of propionate, butyrate or acetate (for example the mean molar proportions of acetate during the infusion period were: 0.591, 0.596, 0.587, 0.599, 0.594, 0.568, 0.616 and 0.606 (sed 0.0129) mMol/ mMol tVFAs for Acid 1, Acid 2, Acid 3, Alkali 1, Alkali 2, Alkali 3, Control 1 and Control 2 respectively). There was a significant effect of treatment upon the molar proportion of isovalerate; the molar proportion of this VFA was markedly higher on Alkali 1 than on the other treatments, during the infusion period the molar proportion were: 0.013, 0.013, 0.012, 0.018, 0.014, 0.013, 0.013 and 0.013 (sed 0.0015) (mMol Isovalerate/ mMol tVFAs) for Acid 1, Acid 2, Acid 3, Alkali 1, Alkali 2, Alkali 3, Control 1 and Control 2 respectively. The plasma concentrations of Na, Cl and CO$_2$ were not affected by treatment, and neither was ruminal concentration of NH$_3$N.

DISCUSSION

The objective of the first experiment reported here was to test whether energy density has any effect upon the diet selections made by sheep. The results from the single feeding treatment, L, M and H have been used to interprete the diet selections of the choice-fed sheep. It is assumed that the sheep on the single food H were able to meet their requirements for energy and protein as they maintained very rapid rates of liveweight (503; sem 28.6, g Lwt/ d), although they had lower rates of food intake than the other single feeding treatments, L and M.
The benefits of eating H must be considered in relation to the possible 'costs' to the animals of eating this food. It is possible that the consumption of food H may have had some adverse effects upon the rumen environment, the rumen wall and the animal's acid-base balance. For example, the rumen liquor pH when food H was offered alone was 5.59 (SEM 0.040); at such a pH cellulolysis would be inhibited (Mould & Ørskov, 1984) and rumen papillae would be clumped (Ørskov, 1973). In addition, foods such as H, which are composed of highly fermentable materials, would be expected to contribute to a high rumen osmolality (Ward et al. 1976). A high rumen osmolality is frequently associated with a decrease in time spent ruminating (Carter & Grovum, 1990a). However the sheep fed food H alone appear to have adapted to cope with the costs of eating H, at least for the duration of the experiment, given the rapid growth rate of the sheep on this food. On the other hand, the consumption of food L appeared to limit the rate of live-weight gain as sheep fed food L had significantly lower rates of live-weight gain than the sheep on H, although the sheep on food L attempted to compensate for the lower energy density of the food by increasing their rate of food intake. It is also likely that the rumen conditions associated with the consumption of L would be less extreme than those associated with food H. No measurements of rumen pH of sheep fed L alone were made, but it can be safely predicted that the rumen pH on food L would be greater than 6.00 (Kaufman, 1976).

From the perspective of a simple theory such as optimal foraging (Krebs & McCleery, 1984), one would expect that sheep offered a choice between a pair of foods of differing estimated ED would show an absolute preference for the more energy dense food, since this choice would enable them to maximise their rate of metabolisable energy intake whilst minimising their intake of dietary bulk (Belovsky, 1978). The diet selections made by the choice-fed sheep in this experiment do not appear to support this hypothesis. The sheep offered the food pairs L/H and M/H did not select food H alone which suggests that, although the sheep had the potential to cope with the costs of eating H, they chose not to adopt this strategy when food H
was offered in a choice. If the diet selections of these animals are expressed as a choice between foods L and H (M was a mixture of foods L and H, 3/4 L: 1/4 H), then the similarities in their dietary choices become more obvious. Castle et al. (1979), Cropper (1987) and Newman et al. (1992) amongst others have also found that the diet selections of ruminants do not appear to follow an 'optimal foraging' strategy.

A few explanations are now offered to account for the behaviour of the choice fed sheep in Experiment 1. The inclusion of a less energy dense food in the diet selected by the L/H and M/H choice-fed sheep may be an example of sampling behaviour, whereby the animal maintains current knowledge of all foods available to it from the environment by eating small quantities of each one at regular intervals (Illius & Gordon, 1990). Herbivores continue to sample foods, even familiar ones in a familiar environment, as their contents of nutrients and anti-nutritive factors may change with time (Provenza & Balph, 1990). The selection of food L by the L/M choice-fed sheep, which accounted for approximately 10-15% of their total intake can be seen as sampling behaviour. However the L/H and M/H choice-fed sheep chose to eat at least a third of their total intake as a food of low energy density; it is unlikely that sampling behaviour alone would account for such a high intake of one of the foods offered in a choice.

A second possibility is that the sheep were selecting on a continuous 'avoidance' basis, whereby they would eat from one food only until some signals (metabolic or otherwise) indicate that there were some adverse consequence associated with the consumption of that food and therefore they would only consume from the other food in a subsequent meal (a theory based on observations on rats (Rozin & Kalat, 1971), but refuted for domesticated animals (Zahorik & Houpt, 1977)). There was no evidence in our experiment that the meals eaten by the sheep consisted exclusively of one of the foods offered or that the composition of the selected diet changed with time. A third explanation of the diet selections made by the L/H
and M/H choice-fed sheep is that they were exhibiting a preference for rarity (Newman et al. 1992). Given the method of presenting the two foods, and as there was little variation in diet selection over the entire experiment, it is improbable that the diet selected by both groups of animals were a result of this.

A fourth and preferred explanation is that maintenance of rumen conditions conducive to rapid rates of cellulolysis and microbial growth as well as the avoidance of conditions such as acidosis and ruminitis is of importance to sheep, and that they will modify their diet selections to maintain an equilibrium within the rumen (as discussed earlier). Given this suggestion, the sheep would be expected to reduce their intake of the food that had greatest potential to change rumen conditions, in this case food H. This hypothesis could also account for the small individual variation in the diet selection of the L/H and M/H choice-fed animals, since it is known that animals vary in their ability to cope with the adverse effects of foods (Kyriazakis & Oldham, 1993). The second experiment was designed to consider the above hypothesis, that is whether the maintenance of rumen conditions (pH and osmolality) has a direct effect upon the diet selection of sheep. The objective of this experiment was to manipulate rumen conditions (pH and osmolality) in the short-term and to observe the effects on the diet selections of sheep. Although fistulated sheep, were used in this experiment it was felt that the conclusions that can be drawn from this experiment can be used to interpret the diet selection made by the sheep in the first experiment.

It is accepted that rumen pH can account for some of the variation in food intake of ruminants (Williams et al. 1987). However, it is likely that the effects of the treatments on food intake in Experiment 2 were mediated by factors other than rumen pH, such as treatment osmolality. Food intake during the time interval in question (1000 - 1600 h) declined systematically in response to increasing treatment osmolality. This result is consistent with the work of a number of other groups (for a review see Carter & Grovum (1990a)), who have also found
that infusions of hypertonic solutions into the rumen are associated with a short-term decline in food intake. There was an apparent effect of the treatment osmolality on food intake after the infusion had ceased (1600 - 0800 h). It would be expected that the influence of treatment osmolality would have been such that sheep with low intakes during the preceding interval (1000h - 1600 h), due in part to the osmotic effects of treatment, would have compensated during the time interval (1600 - 0800 h). The observed direction of the effect of the treatment osmolality upon food intake is opposite to this prediction. In addition the significance of the treatment osmolality upon food intake during the latter time interval appears to have been due to two treatments (Acid 3 and Alkali 3), which were of dissimilar osmolality. This suggests that this effect though statistically significant is not of biological relevance, and so we agree with Phillip et al. (1981) who have shown that the osmolal effect on food intake is of a short term nature only.

There was a significant effect of treatment upon the proportion of food H which was selected by the sheep in Experiment 2 during the time interval from the start of the infusion until two hours after they had ceased (1000 - 1600 h). In this experiment the effect of pH on diet selection cannot be completely separated from osmotic effects. This is because it is not possible to alter rumen pH without affecting osmolality with the acid and alkali treatments used here. However it is thought that the effect of the treatment upon diet selection was mediated through the changes in rumen osmolality that were induced by the infusion of the treatments. In response to increasing treatment osmolality, the intake of food H declined significantly whereas the intake of L remained constant irrespective of treatment osmolality. It is proposed that the decline in the consumption of food H during the infusion period was because this food was composed of materials that would be fermented rapidly within the rumen causing rumen pH to drop and osmolality to rise (Van Soest, 1982). The sheep in Experiment 2 may have been able to associate food H with adverse changes to the rumen environment such as low pH and high osmolality. Thorhallsdottir et al. (1990) have
demonstrated that sheep do have the ability to associate foods with post-ingestive consequences of eating them. In contrast to food H, food L was composed of materials which would be fermented more slowly (Van Soest, 1982); the sheep may have maintained their intake of food L under these conditions as the consumption of this food would generate only a marginal increase in the osmotic load. Engku Azahan & Forbes (1992) have also found that sheep offered a choice between a slowly fermented food (hay) and a rapidly fermented food (barley/dried grass pellets) maintain their intake of hay but reduce their intake of the barley based food, when hypertonic solutions are infused into their rumen. An important finding of this experiment is that although sheep were 'trained' to associate the consumption of the foods with their consequences, their subsequent diet selection was not inflexible but able to respond to short term manipulations of their rumen environment.

It is therefore proposed that sheep in Experiment 2 may have responded to the imposed changes in their rumen environment, in particular increased rumen osmolality, by reducing their intake of food H and in doing so they minimised further increases in rumen osmolality. The process of altering diet selection patterns in response changes to rumen osmolality must be a short-term mechanism, since the sheep were capable of switching between the two foods very rapidly within the four hours of the infusion period. Hou (1991) has also demonstrated that sheep can make fast changes in their diet selection as a result of manipulation of the rumen environment. Carter & Grovum (1990b) have shown that sheep can respond to changes in rumen osmolality very rapidly by reducing their intake within 10 minutes of the start of an rumen infusion of a hypertonic solution. The only receptors that could respond within such a short interval would be those sited in the reticulo-rumen (Carter & Grovum, 1990b). At present the evidence remains equivocal that osmoreceptors do exist in the rumen and that they can be stimulated by changes that are within the physiological range (Forbes & Barrio, 1992).
Tactical adjustments of diet selection in the short-term can be viewed within the overall feeding strategy of the sheep, which is to enable it to maximise its evolutionary fitness. These results suggest that sheep will make short-term changes in diet selection to promote effective rumen conditions for achieving a food intake which allow an animal needs for nutrients and energy to be met. The effect of these shifts in dietary choice would be of benefit to the sheep as they would ensure rapid growth and development over the long term.
Chapter Four

The effects of physical form of food, carbohydrate source and inclusion of sodium bicarbonate, on the diet selections of sheep

3 Submitted to the Journal of Animal Science (November 1994), and therefore American terminology has been used in this chapter to describe the foods used. Authors: Cooper, S. D. B., Kyriazakis, I. & Oldham, J. D.
ABSTRACT

THE EFFECTS OF PHYSICAL FORM OF FOOD, CARBOHYDRATE SOURCE AND INCLUSION OF SODIUM BICARBONATE, ON THE DIET SELECTIONS OF SHEEP

An hypothesis that ruminants attempt to select a diet that promotes optimal rumen conditions has been proposed. Three tests of it were made: is the diet selection of sheep given either a choice of two high energy density (ED) foods or a choice between a high and a low ED food affected by 1) sodium bicarbonate inclusion (NaHCO₃; 1, 2 & 4% (w/w)) in the high (ED) food, 2) dietary carbohydrate source (barley-based, B & sugarbeet/barley-based, S) of the high ED food, and 3) physical form (alfalfa: pelleted, P, & long chop, R) of the low ED food?

To conduct these tests, 42 lambs were used in seven 6x6 Latin squares, which were either foods R and P offered alone and paired with food B or S and their NaHCO₃ derivatives as a choice, or foods B and S offered alone and paired with their NaHCO₃ derivatives as a choice. Each Latin square period lasted for three weeks. The inclusion of NaHCO₃ increased food intake, but there was no effect of level of NaHCO₃ on the proportion of alfalfa (propA) or proportion of food B or S selected. More alfalfa was selected when the high ED was based on barley (B) rather than sugarbeet/barley (S) (p<0.05). PropA selected was greater (p<0.001) when the alfalfa was offered in a pelleted (P) rather than in a long chop form (R). With the exception of a lack of response (in terms of diet selection) of NaHCO₃ level, the other results, namely the effect of dietary carbohydrate source and physical form of the foods on the diet selection, are consistent with the experimental hypothesis. It is suggested that the absence of a response to NaHCO₃ supplementation could reflect the inability of sheep to distinguish between different NaHCO₃ levels due to an insufficient training schedule used for this test.
INTRODUCTION

Sheep offered a choice of foods of high and low energy density (ED) make consistent, non-random selections between the two foods (Cropper, 1987; Parsons et al. 1994). This behaviour is contrary to the expectations that animals make dietary choices to maximise their rate of energy intake (optimal foraging theory, Krebs & McCleery, 1984). Cooper & Kyriazakis (1993), for example, found that sheep selected a diet which tended towards an ME density of around 11.00 MJ ME/kg DM even though a food of substantially higher ME concentration was available. The dietary choices made by sheep in such experiments (Cropper, 1987; Cooper & Kyriazakis, 1993; Parsons et al. 1994) are consistent with the hypothesis proposed by Cooper et al. (1995a) that one of the objectives of dietary choice in ruminants is to maintain rumen conditions within certain physiological limits. The maintenance of rumen conditions that are optimal for the growth of rumen micro-organisms (Hespell & Byrant, 1979) would enable a sustained intake of food and satisfactory yields of energy and protein from the rumen (Theodorou & France, 1993). Such a strategy would assist in the achievement of the goal of meeting requirements for energy and protein. There is some further evidence (Engku Azahan & Forbes, 1992; Cooper et al. 1995a) that sheep will attempt to counteract the effects of direct manipulation of the ruminal environment by adjusting the quantities of foods selected as a choice. In particular, these results suggest that sheep will make selections which have the effect of ensuring the physio-chemical environment in the rumen remains within certain limits. This paper reports the results of three tests of the hypothesis that sheep will adjust selection between foods of different energy density according to the physico-chemical status of the rumen.

The first was to test whether a high ED food supplemented with NaHCO₃ is preferred over the equivalent food left unsupplemented, and whether this preference is affected by either the level of NaHCO₃ supplementation or the source of dietary carbohydrate in the high ED food.
Both Ha et al. (1983) and Erdman (1988) have proposed that NaHCO₃ promotes the intake of high ED foods which would otherwise be associated with a low ruminal pH. It was, therefore, expected that sheep would choose more of the supplemented high ED food than the unsupplemented one, and that this dietary choice would be affected by the level of supplementation such that the sheep would attempt to maintain a constant level of NaHCO₃ in the selected diet. In addition it was proposed that this preference would depend also on the carbohydrate source in the high ED food, as this can have a profound effect upon the rumen environment (Van Soest et al. 1991), perhaps specifically its buffering capacity (McBurnley et al. 1983).

The second test was designed to evaluate the influence of the source of carbohydrate in a high ED food and its selection when offered as a choice with a low ED food, and whether this diet selection would be affected by supplementing the high ED food with NaHCO₃. Williams et al. (1987) have found than foods with a high pectin content (Sugar beet pulp, SBF) are consumed in greater quantities that foods with a high starch content (Barley). Foods based on SBF are associated with a higher ruminal pH than those containing predominantly barley (Rymer & Armstrong, 1989). Therefore it was expected that a greater quantity of high ED food would be selected when it was based upon a material such as SBF rather than when it was predominantly composed of barley. Since these tests were conducted in the presence of different levels of NaHCO₃ in the high ED food, it was further expected that the proportion of high ED food in the selected diet would increase with NaHCO₃ supplementation (up to a certain level of inclusion).

The third test was to determine whether the food intake and diet selection of sheep offered a choice between high and low ED foods is affected when the physical form of the low ED food is manipulated (chopped forage versus pellets). Food intake is increased when low ED foods are physically processed (Owen et al. 1969; Shaver et al. 1986), as these foods can be eaten
more rapidly. However, feeding low ED foods that have been physically processed, for example by grinding and pelleting, reduces salivation (Woodford & Murphy, 1988; Campbell et al. 1992). As a consequence of this, physically processed low ED foods might have a greater impact upon the rumen than the same food offered in an unprocessed form (Block & Shellenberger, 1980; Marshall et al. 1992). When low ED foods with different physical forms are offered as choices with a high ED food, both the potential rate of consumption (Kenney et al. 1984) and the impact of the low ED food upon the rumen environment may influence the selection of low ED food by sheep. In this last test it was expected that the quantity of the high ED food selected would decrease when the low ED food was pelleted rather than chopped. All the above tests were based upon the hypothesis that animals given a choice between foods that promote different physio-chemical conditions in the rumen will attempt to select a diet that ensures that optimal rumen conditions are maintained.

MATERIALS AND METHODS

Animals and Housing

The experimental protocol was reviewed and approved by the Animal Experiments Committee of the Scottish Agricultural College, Edinburgh, Scotland and the procedures used were in accordance with the Animals (Scientific Procedures) Act (1986).

Forty-two female Welsh Mountain sheep were used; they were weaned at 8 wk of age and moved immediately to individual pens in the experimental housing which was naturally ventilated. Each pen measured 1.29 m x 1.53 m and contained one water bucket and two adjustable food troughs; sawdust was used for bedding. During a 3-wk acclimization period, a pelleted food was offered to all sheep (170g crude protein (CP) and 10.01 MJ metabolisable energy (ME) per kg as-fed). The ME content of this food was calculated using the method of Thomas et al. (1988) and was equivalent to 9.45 MJ Net energy (NE) for maintenance/ kg as-
fed and 7.53 MJ NE for gain / kg as-fed (Garrett, 1980). The daily rate of feeding was 4% of mean body weight (BW) of the group. The weight-based allowance was adjusted every week after the sheep had been weighed. It was estimated that this feeding level was just below the voluntary food intake of the majority of the sheep, and would have ensured that all lambs had a similar gut-fill at the point of allocation to a treatment. The experiment lasted from June to November 1993 and the sheep received at least 12 hr of light / d throughout; this was ensured by the use of artificial lighting when necessary. The ambient temperature ranged from 7.8°C (sd 3.02°C) to 15.3°C (sd 3.64°C).

Foods

Four basal foods were used (Table 4:1), differing in their energy density (ED). The foods of low ED consisted of molassed alfalfa (Dengie Crops Ltd., Southminster, Essex, United Kingdom) presented in a long chopped form (Food R; chop length c 120 mm) or a finely ground and pelleted form (Food P). The foods of high ED (B and S) were both pelleted. The ingredients used in Foods B and S were intended to create different ruminal fermentation patterns. To this end, Food B included 64% (as-fed basis) ground barley as a source of rapidly fermentable carbohydrate. In Food S barley was partially replaced by unmolassed SBF, to create a more slowly fermentable carbohydrate matrix. Six other foods were made by supplementing Foods B and S with sodium bicarbonate (NaHCO₃) at inclusion rates of 1, 2 and 4% (w/w); the rate of inclusion is shown in the text by a subscript. Foods B and S were intended to have the same Metabolisable Protein (Agricultural Food Research Council, 1992; MP): ME ratio as Foods R and P (4.0 g MP/Mcal ME). However the calculated MP: ME ratios of the experimental foods as produced differed slightly (for details see Table 4:1); the actual concentration of ME in foods P, R, B, B₄, S and S₄ was determined from a digestibility trial described later. The foods were non-limiting in minerals and vitamins (Agricultural Research Council, 1980). Efforts were made to ensure that the ratios of Ca and
Na to ME were the same across foods, R, P, B and S. The Sodium concentrations of foods B1, B2, B4, S1, S2, S4 were 0.33, 0.61, 1.20, 0.46, 0.74 and 0.95% Na as-fed.

Design

The experiment consisted of seven 6 x 6 Latin squares (six sheep; six feeding treatments), with 21 d. periods. The feeding treatments were either free and continuous access to a single experimental food (single food treatment) or free and continuous access to a choice between two experimental foods (food choice treatment); for details of the all feeding treatments within each Square see Table 4.2. In Squares 1 and 4 the food choice treatments consisted of choices between a high ED food supplemented with NaHCO3 vs. the equivalent high ED food left unsupplemented. The food choice treatments of Squares 2, 3, 5 and 6 consisted of a low ED food that varied in one dimension (physical form) vs. a high ED food that varied in two dimensions (Dietary carbohydrate source and NaHCO3 inclusion level). The experimental hypotheses were: "do sheep show a preference for an NaHCO3 supplemented high ED food over an unsupplemented high ED food, and can this preference be affected by either the level of NaHCO3 supplementation or the source of dietary carbohydrate in the high ED food?" were tested by comparisons made using Squares 1 and 4. The choices offered in these Squares (B and S vs. B1, B2 & B4; S1, S2 & S4 respectively) offered the most distinct tests of the effect of NaHCO3 on diet selection. The second hypothesis was, "does the source of dietary carbohydrate in a high ED food affect selections made between a high and low ED food, and is this choice affected by supplementing the high ED food with NaHCO3?" The last test was, "does the physical form of a low ED food affect the diet selected by sheep from a high and low ED food choice?". Squares 2, 3, 5 & 6 were used in the second and third tests. The purpose of Square 7 was to ensure that the comparisons made between the squares were valid.
### Table 4.1: Composition of the experimental foods

<table>
<thead>
<tr>
<th>Item</th>
<th>R</th>
<th>P</th>
<th>B</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredient composition, % (as-fed basis)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>89.4</td>
<td>89.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Molasses</td>
<td>10.0</td>
<td>10.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Barley</td>
<td>-</td>
<td>-</td>
<td>63.9</td>
<td>32.2</td>
</tr>
<tr>
<td>Unmolassed sugar beet pulp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32.2</td>
</tr>
<tr>
<td>Oatfeed</td>
<td>-</td>
<td>-</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>-</td>
<td>-</td>
<td>21.1</td>
<td>21.2</td>
</tr>
<tr>
<td>Salt</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>Limestone flour</td>
<td>-</td>
<td>-</td>
<td>3.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Calcined magnesite</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
<td>0.4</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mineral and vitamins pre mix^a</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Analysed composition, % (as-fed basis)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>84.2</td>
<td>87.8</td>
<td>86.4</td>
<td>86.5</td>
</tr>
<tr>
<td>ME MJ/kg^b</td>
<td>10.13</td>
<td>9.62</td>
<td>13.31</td>
<td>12.80</td>
</tr>
<tr>
<td>Metabolisable protein (MP)^c</td>
<td>8.54</td>
<td>8.49</td>
<td>11.55</td>
<td>11.03</td>
</tr>
<tr>
<td>MP: ME ratio (g MP/MJ ME)</td>
<td>9.60</td>
<td>8.80</td>
<td>9.20</td>
<td>9.80</td>
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<tr>
<td>Crude protein</td>
<td>13.3</td>
<td>15.3</td>
<td>16.4</td>
<td>15.9</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>45.7</td>
<td>42.2</td>
<td>22.5</td>
<td>29.3</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.17</td>
<td>0.18</td>
<td>0.33</td>
<td>0.36</td>
</tr>
<tr>
<td>Potassium</td>
<td>2.65</td>
<td>2.87</td>
<td>1.12</td>
<td>1.19</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.18</td>
<td>1.51</td>
<td>1.28</td>
<td>1.44</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.23</td>
<td>0.36</td>
<td>0.40</td>
<td>0.36</td>
</tr>
</tbody>
</table>

^a: Pre mix supplied by Scotmin Ltd, Ayr, Scotland. ^b: Calculated from using values obtained from digestibility trial; (pooled SEM=0.071). ^c: Values calculated using the metabolisable protein system (AFRC, 1992), assuming standard values for degradability coefficients.
Table 4.2: The single food and food choice treatments offered to sheep in each experimental Latin Square. The duration of each treatment block was 21 days. The training period of the sheep lasted for two consecutive ten day periods, these were prior to any experimental treatment being given.

<table>
<thead>
<tr>
<th>Square</th>
<th>Treatments</th>
<th>Training period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Blocks)</td>
<td>Period 1</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>P</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>P</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>P</td>
</tr>
</tbody>
</table>

Allocation and training

At the end of the acclimatization period the sheep were allocated to the seven Latin squares, blocked according to their BW. After allocation all sheep had two training periods (i.e. experience of foods to be given as a choice) of ten days each. The use of training in diet selection experiments gives an opportunity for animals to gain experience of each food separately that they will subsequently receive as a choice (Kyriazakis & Oldham, 1993). Sheep are able to associate foods with the post ingestive consequences of eating them (for example Burritt & Provenza, 1991). Also, when a sheep has had prior experience of a new food, this is eaten readily when presented later (Chapple & Lynch, 1986).
During each of the two training periods, two foods were introduced to the sheep in an alternating pattern, this was the same as that used by Cooper et al. (1995a). Details of the foods used in the two training periods for each Square are also given in Table 4:2. It was judged that the sheep would learn quickly about those foods not included in the two training periods (Foods B₁, B₂, S₁ & S₂) using the experiences already gained. Foods R and P were offered in the first training period to all sheep, to ensure some similarities in the training periods across Squares. Foods B and S and their derivatives that were supplemented with NaHCO₃ at 4% (w/w) (B₄ & S₄) were introduced together to ensure that the sheep had the opportunity to make clear distinctions between the supplemented and unsupplemented foods. The quantity of food offered in both training periods was gradually increased from 4% of BW of the individual sheep to ad libitum; this was to ensure that the gastro-intestinal tract of the sheep adapted sufficiently to the foods offered. At the end of the second 10-day training period, all sheep were offered free and continuous access to either the first single food treatment or the first food choice treatment in their allocated Square.

Management and measurements

The positions of the foods in the troughs were randomised across the shed, but it was not changed during the experiment. It has been adequately demonstrated that sheep do not make dietary choices solely on the basis of food position (Cropper, 1987). However in order to maintain continuity within the experiment, when foods R, P, B and S were offered as single food treatments, the food concerned was placed in the same trough as it would have been placed when offered as a food choice.

Food R was prepared daily before feeding, by mixing requisite quantities of monosodium phosphate (0.42% as-fed) and the mineral and vitamin premix (0.20% as-fed) with the long chopped alfalfa; the pre mix and monosodium phosphate adhered readily to the molassed alfalfa. Feeding was twice daily (0800 & 1500 h) to minimise spillage. Food refusals were
collected daily in the morning, before fresh food was offered; the food refusals were weighed and then discarded. Samples of Food R which was refused were composited by sheep, within block and were analysed for Dry matter, DM, Crude protein, CP, Neutral detergent fibre, NDF. Refusals from Food R were treated in this manner as it is known that cattle (and sheep) can select between the stem and leaf fractions of heterogeneous materials such as long chopped alfalfa (Fisher, 1979). It was unnecessary to take such measures with the other foods as they were pelleted. To maintain similar levels of the foods in the troughs, the sheep were offered at least 700g of the pelleted foods and 500g of food R, irrespective of the amounts consumed. The sheep were weighed before feeding on the morning of the first day of each experimental period, and on the same day in subsequent weeks.

*Digestibility trial*

Twenty-four Welsh Mountain sheep from the main experiment were used to measure the digestibility of foods R, P, B, B₄, S and S₄. The sheep were selected for the digestibility trial at the end of the main experiment on the basis of their BW at this time. It was presumed that this would ensure that all chosen sheep would have a similar reticulo-rumen capacity. The sheep were randomly allocated to a food (n=4), after partial balancing for the effects of Square. The trial consisted of a 14 d adaptation period followed by a 10 d balance period, during which faecal and urinary outputs and food refusals were collected. The sheep were offered their allocated foods at a rate estimated to be 90 % of voluntary intake.

Sub-samples of urine and faeces were composited by sheep and were stored in a freezer with samples of the foods. The faecal and food samples were analysed for DM, CP, NDF, and GE. The GE concentration of the urine samples was also measured. Methane production was assumed to be 8% of GE intake (Blaxter, 1967). ME was calculated as DE minus (methane + urinary energy losses).
Statistical analyses

All data from the experiment were analysed using GENSTAT 5.3 (Lawes Agricultural Trust, 1993). The total tract digestibility and ME concentrations of the foods R, P, B, S, B4 and S4 which were determined from the digestibility trial, have been expressed as percentages. A one way analysis of variance was used to test the effect of food with orthogonal contrasts being used to compare: R vs. P, (B & B4) vs. (S & S4), and (B & S) vs. (B4 & S4).

The BW and total food consumption (TFC) data from the main experiment have been expressed as TFC and increases in BW respectively between weeks 1 to 3 (wk1-3) and secondly between weeks 2 to 3 (wk2-3). Food conversion efficiency (FCE, BW gain/TFC) was calculated using data from the same time intervals. The diet selection data were analysed as either the proportion of low ED food selected: food P (Squares 2 and 5) or food R (Squares 3 and 6), or the proportion of unsupplemented high ED food selected; food B (Square 1) or food S (Square 4).

Data from the single food and food choice treatments were analysed separately as incomplete Latin square designs using a Residual Maximum Likelihood (REML; Patterson & Thomson, 1971) procedure. The fixed effect of Food was tested using data from the single food treatments of all seven Squares. Between-square comparisons of the TFC, diet selection, increase in BW and FCE data of the food choice treatments were used to test the three experimental objectives. The data from the food choice treatments were tested for fixed effects of NaHCO3 level, dietary carbohydrate source and physical form; the latter test only included those food choice treatments in which alfalfa was presented as one of the foods offered to the sheep. Wald tests (Lingren, 1976) were used to test the significance of the fixed effect within each respective model.
RESULTS

Digestibility trial

The percentage total tract digestibilities of DM, CP, NDF and the ME concentrations of foods R, P, B and S are presented in Table 4:3. Pelleting the low ED food (molassed alfalfa; Food P) depressed the digestibility of DM by 7% (P<0.01) and the digestibility of NDF by 23.7% (P<0.001). The partial substitution of the barley in Food B with unmolassed SBF to produce Food S, increased the digestibility of NDF significantly from 54.1% to 67.0%; however this partial change in the source of dietary carbohydrate had no significant effect on the digestibility of dry matter. The supplementation of the two high ED foods (B and S) with NaHCO₃ (4% w/w) had no significant effect on either DM digestibility or NDF digestibility. However the inclusion of NaHCO₃ at 4% (w/w) did significantly reduce the ME concentration of both Food B and Food S: ME concentrations of Foods B, B₄, S, S₄ were 13.31, 12.64, 12.76 and 12.30 MJ/kg as-fed respectively.

Performance of the sheep on the single food treatments (data from all squares)

It was necessary to exclude the data from one lamb from Square 3 for one period; this animal was ill for two weeks of that period, but subsequently made a complete recovery. No other problems were incurred and all sheep completed the 18 weeks of the experiment.

The changes in BW, food conversion efficiency (FCE) and total food consumption (TFC) of the sheep given access to the single foods R, P, B and S are given in Table 4:4. It was expected that the food intake of the sheep would change during week 1, as the digestive tracts of the animals became adapted to a change of feeding treatment. The diet selections made by the sheep on food choices were also expected to alter during the initial week as the sheep became familiar with the dietary options. Accordingly, the BW, FCE, TFC and diet selection
(where appropriate) results have been expressed in two ways, the first has included data from week 1-3 and the second has used data from week 2-3 only.

Table 4.3: Percentage whole tract digestibility of dry matter, crude protein and neutral detergent fibre of the Experimental foods R, P, B, B₄, S & S₄.

<table>
<thead>
<tr>
<th>Food</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Neutral detergent fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>63.8</td>
<td>62.7</td>
<td>61.1</td>
</tr>
<tr>
<td>P</td>
<td>59.5</td>
<td>65.8</td>
<td>46.7</td>
</tr>
<tr>
<td>B</td>
<td>78.6</td>
<td>81.0</td>
<td>54.1</td>
</tr>
<tr>
<td>B₄</td>
<td>79.1</td>
<td>81.0</td>
<td>52.0</td>
</tr>
<tr>
<td>S</td>
<td>77.1</td>
<td>78.8</td>
<td>67.0</td>
</tr>
<tr>
<td>S₄</td>
<td>79.8</td>
<td>80.9</td>
<td>73.7</td>
</tr>
</tbody>
</table>

SED 1.40 .1.78 2.78

Contrasts

R vs P ** NS ***
B vs S NS NS **
NaHCO₃(0 vs 4%) NS NS NS

Consumption of food P was greater than the other single food treatments over week 2-3. On food S the increase in BW over week 1-3 was greater (p<0.05) than on the other single food treatments, although the effect was less clear (0.1<p<0.05) over the week 2-3 interval. Over the whole experimental period FCE was lower on foods P and R (P<0.001). The NDF and CP concentrations (dry matter basis) of the food R that was left unconsumed were similar to the NDF and CP concentrations (dry matter basis) of the food R offered, which suggests that the sheep did not exhibit any selection within this food.
Table 4:4: Influences on BW, total food consumption (TFC) and food conversion efficiency (FCE) on the single food treatments of all seven Squares. For details of the foods see Table 4:1; all the treatments are listed in Table 4:2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW increase (kg)</th>
<th>TFC(kg)</th>
<th>FCE(kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wk1-3\textsuperscript{a}</td>
<td>wk2-3\textsuperscript{b}</td>
<td>wk1-3\textsuperscript{a}</td>
</tr>
<tr>
<td>R</td>
<td>2.12</td>
<td>1.66</td>
<td>21.75</td>
</tr>
<tr>
<td>P</td>
<td>2.39</td>
<td>1.76</td>
<td>24.56</td>
</tr>
<tr>
<td>B</td>
<td>2.17</td>
<td>1.99</td>
<td>20.12</td>
</tr>
<tr>
<td>S</td>
<td>2.80</td>
<td>2.27</td>
<td>21.70</td>
</tr>
<tr>
<td>SED</td>
<td>0.348</td>
<td>0.289</td>
<td>1.357</td>
</tr>
</tbody>
</table>

Food effects:

| Food | * | † | NS | *** | *** | *** |

\textsuperscript{a}: includes data from weeks 1-3 of each experimental block; \textsuperscript{b}: includes data from weeks 2-3 of each experimental block; †: P value within the range 0.05<p<0.1, ***: P<0.001

**Performance and diet selection of the sheep given a food choice - Squares 1 & 4**

The increases in BW, FCE and TFC of the sheep on the food choice treatments of Square 1 (Food B vs. B\textsubscript{1}, B\textsubscript{2} and B\textsubscript{4}) and Square 4 (Food S vs. S\textsubscript{1}, S\textsubscript{2}, and S\textsubscript{4}) are shown in Table 4:5. The diet selections made by the sheep on these treatments have been expressed as the weight of unsupplemented food (B or S) that was selected per kg TFC. The main effect of dietary carbohydrate source (Square 1 vs. Square 4) and NaHCO\textsubscript{3} level are shown; there were no interactions between these two factors.

There was no effect of dietary carbohydrate or NaHCO\textsubscript{3} level on either BW gain or FCE. The sheep on the SBF/barley choices (S series) tended to have higher levels of TFC than those on the barley-based choices (B series; p<0.1). However, food consumption was not affected by the levels of NaHCO\textsubscript{3} inclusion.
Over wk1-3 the proportion of unsupplemented food selected on both Squares was affected
(p<0.05) by NaHCO₃ level; this effect was not dose dependent. However, diets selected by
the sheep during wk2-3 were not significantly affected by NaHCO₃ level. There was no
significant effect of dietary carbohydrate source on the dietary choices of the sheep in these
two Squares as the proportions of unsupplemented food selected on the choice-feeding
treatments of barley (B) based choices were not significantly different from those made on the
equivalent SBF/barley (S) based choices.

Table 4:5: Influences on BW, food conversion efficiency (FCE), total food consumption
(TFC) and diet selection (g unsupplemented food (B or S) per kg TFC) of the lambs given
choices between NaHCO₃ supplemented and unsupplemented high ED foods. For details
of the foods see Table 4:1; all the food choices in each Square as listed in Table 4:2.

<table>
<thead>
<tr>
<th>BW increase (kg)</th>
<th>FCE (kg/kg)</th>
<th>TFC (kg)</th>
<th>Diet selection (g B or S/kg TFC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk1-3a</td>
<td>wk2-3b</td>
<td>wk1-3a</td>
<td>wk2-3b</td>
</tr>
<tr>
<td>wk1-3a</td>
<td>wk2-3b</td>
<td>wk1-3a</td>
<td>wk2-3b</td>
</tr>
</tbody>
</table>

Square 1

<table>
<thead>
<tr>
<th></th>
<th>B/B₁</th>
<th>B/B₂</th>
<th>B/B₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/B₁</td>
<td>3.53</td>
<td>3.17</td>
<td>3.78</td>
</tr>
<tr>
<td>B/B₂</td>
<td>2.91</td>
<td>2.55</td>
<td>2.60</td>
</tr>
<tr>
<td>B/B₄</td>
<td>0.160</td>
<td>0.147</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>0.190</td>
<td>0.176</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>22.06</td>
<td>21.54</td>
<td>22.90</td>
</tr>
<tr>
<td></td>
<td>15.27</td>
<td>14.51</td>
<td>15.37</td>
</tr>
<tr>
<td></td>
<td>461</td>
<td>363</td>
<td>608</td>
</tr>
<tr>
<td></td>
<td>433</td>
<td>437</td>
<td>629</td>
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Square 4

<table>
<thead>
<tr>
<th></th>
<th>S/S₁</th>
<th>S/S₂</th>
<th>S/S₄</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/S₁</td>
<td>3.29</td>
<td>3.38</td>
<td>3.96</td>
<td>0.671</td>
</tr>
<tr>
<td>S/S₂</td>
<td>2.21</td>
<td>2.12</td>
<td>2.65</td>
<td>0.649</td>
</tr>
<tr>
<td>S/S₄</td>
<td>0.137</td>
<td>0.142</td>
<td>0.166</td>
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</tr>
<tr>
<td></td>
<td>0.142</td>
<td>0.140</td>
<td>0.161</td>
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</tr>
<tr>
<td></td>
<td>24.00</td>
<td>23.75</td>
<td>24.40</td>
<td>1.679</td>
</tr>
<tr>
<td></td>
<td>15.51</td>
<td>15.15</td>
<td>16.45</td>
<td>1.715</td>
</tr>
<tr>
<td></td>
<td>335</td>
<td>276</td>
<td>375</td>
<td>109</td>
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<tr>
<td></td>
<td>359</td>
<td>290</td>
<td>382</td>
<td>121</td>
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</tbody>
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Effects

<table>
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<tr>
<th></th>
<th>Carb</th>
<th>HCO₃</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NS</td>
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<table>
<thead>
<tr>
<th></th>
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<th>†</th>
<th>NS</th>
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<table>
<thead>
<tr>
<th></th>
<th>†</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>include data from weeks 1-3 of each experimental block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>† P value within the range 0.05&lt;p&lt;0.1</td>
</tr>
<tr>
<td>*</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>
Performance and diet selection of the sheep given a food choice - Square 2, 3, 5 and 6

The increases in BW, FCE, TFC and diet selection of the sheep on the Barley based food choice treatments of Square 2 (P vs. B, B1, B2, B4 (B series)), Square 3 (R vs. B series), and the SBF/Barley-based food choices of Square 5 (P vs. S, S1, S2, S4 (S series)) and Square 6 (R vs. S series) are shown in Table 4:6. The diet selections made by the sheep have been expressed as the weight of alfalfa (Food P or Food R) selected per kg TFC. The main effects of physical form (pelleted, P vs. long chop, R), dietary carbohydrate source and NaHCO$_3$ level are shown. The significance of the interaction between physical form and carbohydrate source is also shown. No other interactions are shown as they were not significant.

The BW gains over wk1-3 and wk2-3 were higher (P<0.05) when food R rather than P was paired with the S series (Square 5) vs (Square 6). However there were no differences in BW gain between the two physical forms of alfalfa when paired with the B series of foods. The effect of physical form on FCE was similarly affected (p<0.05) by the source of dietary carbohydrate. There was no effect of NaHCO$_3$ level on the TFC of the sheep. In addition, TFC was not affected by either physical form or the source of dietary carbohydrate.

Irrespective of the source of dietary carbohydrate in the high ED food, the sheep selected more alfalfa when it was pelleted (food P) than when it was available in a long chop form (Food R) (379 vs 199 g/kg TFC (SED 32.5; p<0.001)) (Figure 4:1). A greater quantity of alfalfa was selected on the barley-based choices (P<0.05) than on the SBF/barley choices in weeks 1-3 (321 vs 257 g/kg TFC (SED 32.5) respectively). This effect of source of dietary carbohydrate on the proportion of alfalfa selected by the sheep tended to persist during the interval week 2-3 (Figure 4:1).
Table 4:6: Influences on BW, food conversion efficiency (FCE), total food consumption (TFC) and diet selection (g of alfalfa selected per kg TFC) on the choice feeding treatments of Squares 2, 3, 4 and 6. For details of the foods see Table 4:1; all the treatments in each Squares are listed in Table 4:2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BW increase (g)</th>
<th>FCE (kg/kg)</th>
<th>TFC (kg)</th>
<th>Diet selection (g P or R/kg TFC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wk1-3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>wk2-3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>wk1-3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>wk2-3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><strong>Square 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P/B</td>
<td>2.93</td>
<td>2.40</td>
<td>0.117</td>
<td>0.143</td>
</tr>
<tr>
<td>P/B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.35</td>
<td>2.91</td>
<td>0.130</td>
<td>0.168</td>
</tr>
<tr>
<td>P/B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.63</td>
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<td>0.130</td>
<td>0.148</td>
</tr>
<tr>
<td>P/B&lt;sub&gt;4&lt;/sub&gt;</td>
<td>3.68</td>
<td>2.70</td>
<td>0.127</td>
<td>0.146</td>
</tr>
<tr>
<td><strong>Square 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/B</td>
<td>3.44</td>
<td>2.55</td>
<td>0.137</td>
<td>0.150</td>
</tr>
<tr>
<td>R/B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.11</td>
<td>1.84</td>
<td>0.129</td>
<td>0.116</td>
</tr>
<tr>
<td>R/B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.82</td>
<td>2.55</td>
<td>0.161</td>
<td>0.158</td>
</tr>
<tr>
<td>R/B&lt;sub&gt;4&lt;/sub&gt;</td>
<td>3.13</td>
<td>2.51</td>
<td>0.129</td>
<td>0.155</td>
</tr>
<tr>
<td><strong>Square 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P/S</td>
<td>2.28</td>
<td>1.55</td>
<td>0.094</td>
<td>0.099</td>
</tr>
<tr>
<td>P/S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2.21</td>
<td>1.53</td>
<td>0.100</td>
<td>0.105</td>
</tr>
<tr>
<td>P/S&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>2.18</td>
<td>0.134</td>
<td>0.138</td>
</tr>
<tr>
<td>P/S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>3.00</td>
<td>1.85</td>
<td>0.121</td>
<td>0.109</td>
</tr>
<tr>
<td><strong>Square 6</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/S</td>
<td>3.23</td>
<td>2.58</td>
<td>0.135</td>
<td>0.158</td>
</tr>
<tr>
<td>R/S&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>2.43</td>
<td>0.146</td>
<td>0.143</td>
</tr>
<tr>
<td>R/S&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>2.36</td>
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<td>0.146</td>
</tr>
<tr>
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<td>4.79</td>
<td>3.70</td>
<td>0.180</td>
<td>0.208</td>
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<tr>
<td><strong>SED</strong></td>
<td>0.709</td>
<td>0.767</td>
<td>0.0264</td>
<td>0.0442</td>
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</tbody>
</table>

Treatment effects

| Physical form (P) | NS | NS | NS | NS | NS | NS | *** | *** |
| Carb (C) | NS | NS | NS | NS | NS | NS | * | |
| HCO<sub>3</sub> | NS | NS | NS | NS | NS | NS | NS | NS |
| P x C | ** | ** | ** | ** | NS | NS | NS | NS |

<sup>a</sup> Includes data from weeks 1 to 3 of each experimental block.<br><sup>b</sup> Includes data from weeks 2 to 3 of each experimental block. <sup>†</sup> P value within the range 0.05<p<0.1. <sup>**, ***</sup>, p<0.05, p<0.01, p<0.001 respectively.
Figure 4:1 Diet selection of the sheep (g/alfalfa per kg total food consumption). (Bar refers to standard error). For details of foods see Table 4:1.
Performance and diet selection on the food choices R/P and B/S

Over the interval wk1-3 the BW gain and TFC of the sheep on the food choice B/S were not significantly different from the sheep on the single foods B and S; BW gains were: 2.32, 2.17 and 2.80 (SED 0.525) kg; TFC: 21.32, 20.12 and 21.70 (SED 1.101) kg for treatments B/S, B and S respectively. When data from week 1 were excluded from the analysis the sheep on the choice B/S grew less rapidly than those on the single foods B or S: 1.34, 1.99, 2.27 (sed 0.470) (p<0.05) and as a consequence the FCE of the sheep on B/S tended to be lower than that of the sheep on either B or S alone: 0.099, 0.147, 0.155 (SED 0.0338) kg gain/kg food respectively. Over both time intervals the BW gain and FCE of the sheep on the food choice R/P was not significantly different from the sheep on the single foods P and R. Total food consumption on R/P was significantly greater than on food R, TFC (wk1-3): 25.92, 20.12, 24.56 (SED 1.41) (p<0.05) for treatments R/P, R and P respectively. The proportion of B selected by the sheep on the choice B/S was 431 (se 93.5) g food B/kg TFC, and the proportion of R selected was 270 (se 10.5) g food R/kg TFC on the choice P/R.

DISCUSSION

The purpose of this work was to conduct three tests of the hypothesis proposed by Cooper et al. (1995a), namely that one of the objectives of diet selection in ruminants is to maintain optimal rumen conditions, or to at least keep them within certain physiological limits. Emmans (1991) suggested that the design of diet selection experiments of this nature would be strengthened if, in addition to the food choice treatments, like animals were offered the foods which were included in food choice treatments as single foods. The results obtained from such single food treatments can be used to interpret the diet selections made by the sheep offered food choices (Kenney et al. 1984; Kyriazakis & Oldham, 1993).
Single food treatments

When the low ED food (molassed alfalfa) was offered as a single food, it was eaten in greater quantities when it was pelleted (Food P) than when it was offered in a long chop form (food R). It is generally accepted that the physical processing of low ED foods enables the rate of passage through the rumen to be increased, with the result that ruminants fed pelleted forages generally eat more than those offered the same material left unprocessed (Owen et al. 1969; Shaver et al. 1986). However the consumption of physically processed forages, such as Food P is also associated with reduced levels of mastication and salivation (Campbell et al. 1992), with the result that ruminal pH is reduced (Marshall et al. 1992) and ruminal osmolality is increased (Teller et al. 1989).

Sheep offered food S alone consumed greater amounts of food than those on the other high ED food, B. It may be that food S could be eaten in greater quantities because its consumption had a lesser impact upon the rumen environment than food B, due to the different dietary sources of carbohydrate present in foods B and S. Food S was based on equal parts mixture of unmolassed sugar beet pulp (SBF) and barley, whereas food B was composed of barley predominantly. Foods which contain a considerable quantity of SBF are associated with a higher ruminal pH than foods with a higher barley content (Rymer & Armstrong, 1989), which is a consequence of the higher buffering capacity of SBF (McBurnley et al. 1983). It might also be expected that the consumption of S would yield a different pattern of fermentation than when food B was consumed (Sutton et al. 1986), with S yielding a lower ruminal lactic acid concentration than food B, due to the high pectin content of SBF (Van Soest et al. 1991). Both a high level of lactic acid and a low ruminal pH reduce the efficiency of ruminal microorganisms (Mackie et al. 1978; Russel & Strobel, 1993) and reduce the food intake of the host ruminant (Williams et al. 1987). It is therefore likely that the sheep on food B grew less rapidly than those on food S because their intake was limited by physiological factors.
Food choice treatments

On the food choice treatments it was expected that the foods supplemented with NaHCO₃ would be chosen in greater quantities than the unsupplemented ones; this preference was expected to be affected by both the level of NaHCO₃ supplementation and the dietary carbohydrate source. This prediction was based on the fact that the inclusion of NaHCO₃ can stimulate the intake of high ED foods (for a review see Erdman, 1988). This response is thought to be mediated through an increase in rumen liquor pH (Ha et al. 1983), but this relationship is not always evident (Hart & Doyle, 1985).

During the interval wk2-3 the food intake of the sheep on the food choice S/S₁ was increased relative to those on food S alone, but this increase was marginal in comparison with the increase in food intake on food choice B/B₁ relative to food B. Marked responses in food intake to NaHCO₃ inclusion have only been demonstrated when food intake on the equivalent unsupplemented food was depressed (Hart & Polan, 1984). This difference in response to the inclusion of NaHCO₃ at a rate of 1% (w/w) may reflect differences in the intrinsic buffering capacity of the ingredients that were contained in food B and S (Van Soest et al. 1991). However, when higher levels of NaHCO₃ were added to food B (2 and 4% (w/w), B/B₂ & B/B₄), there were no further increases in food intake or BW gain. This indicates that when the level of NaHCO₃ was greater than 1% (w/w), it did not have a net positive effect on the performance of the sheep. To add to this, Hart & Polan (1984) have found that the BW gain of ruminating calves is maximised when the inclusion of NaHCO₃ is between 1-2% (w/w).

The question then is why sheep did not modify their diet selection as the NaHCO₃ content of the supplemented foods was increased? By decreasing the proportion of the supplemented food in their diet they could have achieved NaHCO₃ intakes similar to the ones achieved on the B/B₁ or S/S₁ choices. There was only a transient effect of NaHCO₃ level on the proportion of unsupplemented high ED food selected. However this effect was not present if
data from week 1 are excluded. This effect of NaHCO₃ level on dietary choice may be spurious, and could be attributed to increased sampling behaviour when novel foods are offered to animals, including ruminants (Illius & Gordon, 1990). The absence of a dose-dependant response to the level of NaHCO₃ supplementation on dietary choice may indicate that the sheep were unable to discriminate between the three levels of NaHCO₃ inclusion and therefore treated them similarly when offered in a food choice. This may be because the training regime used in this experiment may not have been appropriate for this test of the experimental hypothesis. It is also possible that the changes induced in the rumen by the quantities of NaHCO₃ that were ingested on the different levels of supplementation were not sufficiently distinct that they would force the sheep to alter their diet selections; however this hypothesis could not be tested as no direct measurements of the rumen environment were made.

Previous experiments have shown that sheep given a choice between a high- and low-ED food select a substantial quantity of the low-ED food (Cropper, 1987; Cooper & Kyriazakis, 1993). The second aim of this work was to investigate whether the source of dietary carbohydrate in a high-ED food would affect its selection by sheep, when it is offered as a choice with a low-ED food, and whether this choice was affected by the supplementation of the high-ED food with NaHCO₃. On the basis of predicted differences in the buffering capacity and ruminal fermentation pattern of foods B and S (McBurnley et al. 1983; Sutton et al. 1986) it was expected that a greater proportion of low-ED food would be selected on the barley-based (B) choices than on the SBF/barley (S) choices and that the proportion of low-ED food would decline in response to supplementing the high-ED food with NaHCO₃.

The results of the experiment show that a higher proportion of the low-ED food was selected when the other food on offer was S rather than B. However, when the foods B and S were paired with the low ED foods, the proportion of low-ED food selected was similar irrespective
of whether the high-ED food in the choices were supplemented with NaHCO₃ at 1, 2 and 4% (w/w) or were left unsupplemented. The concept advanced earlier to account for an absence of response (in terms of diet selection) to the NaHCO₃ supplementation could partly be invoked here. Alternatively, because the low-ED food was based on alfalfa, which has a high buffering capacity (McBurnley et al. 1983), its inclusion in the selected diet may have had an overwhelming influence on rumen conditions such that intake of NaHCO₃ would not be perceived by the animal as having any major influence. Hadjipanayiotou (1982) and Mould & Ørskov (1984) have found that the addition of roughage to a cereal-based food is more effective than NaHCO₃ at maintaining rumen conditions. The efficacy of alfalfa as a buffering agent may account for the similarity of diet selections made across NaHCO₃ levels.

One of the few effects on the performances of the animals in this experiment was that seen in the P vs S series choices. In this specific case when food P was offered as a choice with food S or S₁, there was a particularly low efficiency of utilisation, and slow growth. These poor performances accounted entirely for the apparent interaction between physical form and dietary carbohydrate on BW gain. We do not have any particular explanation to offer for this result; it cannot be accounted for by their diet selection (P and S foods were eaten in quantities similar to the other food choices that included a low-ED food).

The third element of this work was to determine whether the physical form of the low-ED food has an effect upon the diet selection of sheep offered a choice between high-and low-ED foods. Kenney et al. (1984) have found that sheep will select the food which has the greatest potential intake rate, when they are offered a choice between foods (straw or hay) of different chop length. Such a selection would be consistent with optimal foraging theory (Krebs & McCleery, 1984). Pelleted low ED foods can be eaten more rapidly than the same food left unprocessed, thus it would be expected that sheep offered foods P and R would choose a diet that was composed entirely or at least predominantly of food P. However sheep offered foods
P and R as a choice did not consume food P solely; instead they included a substantial amount of food R in their selected diets (27% of their diet), and this cannot be explained simply in terms of a sampling behaviour.

The differences in the physical forms of foods P and R were more marked than the differences in the physical forms of the foods used by Kenney et al. (1984). As a consequence of this, factors other than potential intake rate may have affected the dietary choice of the sheep in our experiment. The production of saliva is reduced substantially when low-ED foods are physically processed (Block & Shellenberger, 1980; Campbell et al. 1992), with the result that consuming food P would be expected to have a greater impact on the rumen environment than consuming food R, as saliva is both a buffering agent and a diluent (Carter & Grovum, 1990a). It is proposed that the sheep on the food choice P/R chose to include substantial amounts of food R in their selected diets in an attempt to maintain certain rumen conditions.

When the low-ED foods were offered to sheep as paired choices with either food B or food S, a greater proportion of alfalfa was selected when it was presented as food P than when it was left in a roughage form, as food R. The effect of physical form on the dietary choice of sheep could be solely due to differences in the potential intake rate on foods R and P. Alternatively, a higher amount of food P may be needed in the selected diet to equate in same terms with an amount of food R consumed when either food is given a choice with the high ED food. If we were to take this view then the perceived attribute of R is about twice that of P. This clearly would not be accountable in terms of conventional chemical attributes (Table 4:1), however this attribute may be quantified by a biological index. Indexing of physical attributes of roughages, for example through the use of time spent chewing (Balch, 1971) remains a challenge. The selections made by the sheep on the choice P/R indicate that while differences in potential intake rate can affect dietary choice (Kenney et al. 1984), alternative strategies might also be important, possibly to maintain optimal ruminal conditions. This is the first
indication, to our knowledge, that the physical form of a low-ED food can have an effect on the diet selection of sheep from a choice of a low- and a high-ED foods. Further experiments could use less dramatic differences in the form of the low-ED food (e.g., chopped roughage of various lengths) to test whether functional physical properties are important in selection decisions.

In conclusion, the results suggest that, although the inclusion of NaHCO₃, which could increase ruminal pH, increases the intake of high ED foods, neither the level of NaHCO₃ in a supplemented high-ED food, nor the carbohydrate source of such a food have an effect on the dietary choices made by sheep given a choice between supplemented and unsupplemented high ED foods. Secondly, the nature of the carbohydrate in a high ED food does appear to affect the proportion of high ED food selected when presented as a choice with a lower ED food. Lastly differences in the potential intake rate and the functional physical attributes of low ED foods may have an effect upon the diet selections made between high and low ED foods. The results of this work are consistent with the hypothesis that one of the objectives of diet selection in sheep is to maintain optimal rumen conditions or at least to keep them within certain physiological limits.
Chapter Five

Changes in the feeding behaviour of sheep given a choice between two foods that differ in physico-chemical characteristics, in response to a challenge to their rumen environment.

Submitted to Appetite, (February 1995)

Authors: Cooper, S. D. B., Kyriazakis, I., Oldham, J. D. & Anderson, D. H.
ABSTRACT

CHANGES IN THE FEEDING BEHAVIOUR OF SHEEP GIVEN A CHOICE BETWEEN TWO FOODS THAT DIFFER IN PHYSICO-CHEMICAL CHARACTERISTICS, IN RESPONSE TO A CHALLENGE TO THEIR RUMEN ENVIRONMENT

The objective of the experiment was to test the hypothesis that sheep will respond by altering their diet selection to minimise the effect of disturbances to the rumen environment, and secondly that the nature of the food choice offered would affect the nature of the change in diet selection. Two low-energy dense (ED) foods (9.95 MJ/kg food): R (long chop) P (pelleted) and a high-ED food, B (13.4 MJ ME/kg food) were offered ad libitum singly or as choices (B/R, B/P & P/R) to rumen-fistulated sheep. The design was three 3 x 3 Latin Squares with 21 d. periods; treatments within squares consisted of two single foods and their respective choices. Days 1-7 were allowed for adaptation; during days 15-17 the sheep received a rumen infusion of a mineral acid (11. 400mM HCl, 1000-1400 h). Food intake (FI) was measured every 2 h (0800-1600 h) on days 12-13 (Control), days 16-17 (Infusion) and on days 20-21 (Post-infusion). Rumen pH and osmolality were measured at the same two-hourly intervals on day 13 (Control), day 17 (Infusion) and day 21 (Post-infusion). During the Control sub-period rumen pH on food R was higher and osmolality lower than on the foods P and B (p<0.05). The infusion during the first 2-h interval (1000-1200 h) caused rumen pH to decline, and the proportion of B or P on the three choices to be reduced (p<0.05) relative to the Control and Post-infusion sub-periods; food intake and rumen osmolality were unaffected. Between 1200-1400 h, the infusion maintained a low rumen pH and food intake declined relative to Control and Post-infusion sub-periods (this was most marked on choice B/P); there were no further changes to diet selection during the Infusion sub-period. These results suggest that the first response of sheep to a challenge to the rumen environment is to alter their diet selection to
minimise the induced imbalance. However, if the nature of the food choice offered does not permit such a response, sheep will reduce their food intake.

INTRODUCTION

The purpose of the experiment reported here was to draw together the results of two previous experiments (Cooper et al. 1995a; Cooper et al. 1995b) that studied the relationship between the rumen environment and the diet selection of sheep. The dietary choices made by sheep were studied to determine whether these selections would change in response to a challenge to the rumen environment, and to determine whether the nature of the food choice offered to sheep has an effect upon such a response. We have demonstrated (Cooper et al. 1995a) that sheep which are given a choice between foods of high- and low-energy density (ED) respond to direct challenges to the rumen environment, by reducing the intake of a high-ED food in the short term. This change in dietary choice was consistent with the above hypothesis, as the consumption of a high ED food is generally associated with a reduced rumen pH (Counette et al. 1979) and increased rumen osmolality (Carter & Grovum, 1990a); such conditions would compromise an animal's ability to consume sufficient food to meet its requirements.

The second aspect to be evaluated was whether the physico-chemical attributes of foods offered in a choice affect the response made by sheep, when the rumen environment is challenged directly. In general, feeding low-ED foods, which have been physically processed, for example by grinding and pelleting, leads to a decrease in chewing activity, and therefore salivation (Woodford & Murphy, 1988; Campbell et al. 1992). As a consequence of this physically processed foods may have a greater impact upon the rumen environment, than the same food left unprocessed (Block & Shellenberger, 1980); for example rumen osmolality is increased on physically processed foods (Teller et al. 1989) and rumen pH is reduced (Marshall et al. 1992). We have previously suggested that differences in the physical
attributes of low ED foods may have an effect upon the dietary choices made by sheep, between high and low ED foods (Cooper et al. 1995b). It was proposed that when the rumen environment is directly challenged, sheep which are offered such a food choice will reduce the intake of the food which has the greater potential to adversely alter rumen conditions.

The main objective of the present experiment was to test the hypothesis that, if the physico-chemical environment of the rumen is directly challenged through the administration of an acid solution, sheep would attempt to select a diet that would 'correct' imbalances that have been created, and that the nature of the food choice offered to sheep would have an effect upon the response made.

MATERIALS AND METHODS

Animals, housing and foods

Animals & housing
Nine rumen-cannulated female Texel x Scottish Blackface sheep (21 months old) and weighing 71.0 kg (sd 4.4) were used and housed in metabolism crates. These were placed in a naturally ventilated room, which has been described previously (Cooper et al. 1995a). The sheep had received prior experience of both the experimental environment and procedures (Cooper et al. 1995a). An artificial lighting system was used in the relatively light-proof room to ensure that the sheep received light between 0700-1900 h throughout the experiment (26 January-11 May 1994).

Foods
Three foods were used (Table 5:1). Two were of a low energy density; these consisted of molassed alfalfa (Dengie Crops Ltd., Southminster, Essex, United Kingdom) presented in two physical forms, either in a long-chop form (food R; chop length c120 mm) or in a finely
ground and pelleted form (food P). It was intended that the same source of alfalfa was to be used to produce foods R and P. Food R was prepared daily before feeding by mixing the requisite quantities of monosodium phosphate and the mineral pre-mix with the molassed long chop alfalfa; these materials adhered together readily (this method was previously used by Cooper et al. 1995b).

The third food was a high ED food (B), which was composed predominately of ground barley (63.9 %). It was expected that food B would be fermented rapidly in the rumen, yielding a fermentation pattern which would differ from that which would be produced from either of the alfalfa foods (Sutton et al. 1986). The three experimental foods were the same as those which were fed to young, growing sheep in a previous experiment (Cooper et al. 1995b). Food B was intended to have the same ratio of metabolisable protein (Agricultural and Food Research Council; MP):metabolisable energy (ME) as the two alfalfa foods P and R (8.0 g MP/ MJ ME). However, there was a slight difference in the calculated MP:ME ratios of the three foods that were produced (Table 5:1). The foods were intended to be non-limiting in minerals and vitamins (Agricultural Research Council, 1980) and maintained a constant ratio of macro minerals to ME.

Design

The experiment consisted of three 3 x 3 Latin squares (3 sheep; 3 feeding treatments in each square) with 21-day periods. The sheep were randomly allocated to the three squares at the beginning of the experiment. The sheep were taken out of metabolism crates during days 1-7 of the third period, in accordance with Home Office guidelines (Animals (Scientific Procedures) Act (1986)), which state that animals are not expected to remain in metabolism crates for a period of longer than 6 weeks.
### Table 5.1: Ingredients and chemical composition of the experimental foods

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<th>Ingredients (g/kg)</th>
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<th>P</th>
<th>B</th>
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<td>894.0</td>
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</tr>
<tr>
<td>Molasses</td>
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<td>100.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Barley</td>
<td>-</td>
<td>-</td>
<td>639.0</td>
</tr>
<tr>
<td>Oatfeed</td>
<td>-</td>
<td>-</td>
<td>58.0</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>-</td>
<td>-</td>
<td>211.0</td>
</tr>
<tr>
<td>Salt</td>
<td>-</td>
<td>-</td>
<td>6.3</td>
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<tr>
<td>Limestone flour</td>
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<td>Calcined magnesite</td>
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<td>2.5</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
<td>4.2</td>
<td>4.2</td>
<td>-</td>
</tr>
<tr>
<td>Mineral &amp; vitamins pre-mix\textsuperscript{a}</td>
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</table>

#### Chemical analyses (g/kg fresh matter unless otherwise stated)

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<tr>
<th></th>
<th>R</th>
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<td>864.0</td>
</tr>
<tr>
<td>Metabolisable energy (ME; MJ/kg)\textsuperscript{b}</td>
<td>10.3</td>
<td>9.8</td>
<td>13.6</td>
</tr>
<tr>
<td>Metabolisable protein (MP)\textsuperscript{c}</td>
<td>85.4</td>
<td>84.9</td>
<td>115.5</td>
</tr>
<tr>
<td>MP:ME (gMP/MJ ME)</td>
<td>8.3</td>
<td>8.6</td>
<td>8.5</td>
</tr>
<tr>
<td>Crude protein</td>
<td>133.1</td>
<td>153.4</td>
<td>164.2</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>457.2</td>
<td>422.4</td>
<td>300.9</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.7</td>
<td>1.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Potassium</td>
<td>26.5</td>
<td>28.7</td>
<td>11.2</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.8</td>
<td>15.1</td>
<td>12.8</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.3</td>
<td>3.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}: Pre-mix supplied by Scotmin Ltd, Ayr, Scotland. \textsuperscript{b}: Calculated from values obtained from digestibility trial. \textsuperscript{c}: Values calculated using the metabolisable protein system (AFRC, 1992), assuming standard values for degradability coefficients.
In two of the three feeding treatments the sheep had free and continuous access to one of the experimental foods (single food treatment); in the third treatment the sheep had free and continuous access to a choice between the two foods (food choice treatment). The food choice treatment in each square was either the high ED feed paired with one of the two low ED foods (B/R and B/P) or the two low ED foods offered together (P/R) as a choice.

The hypotheses tested were 1) whether the dietary choices made by sheep would change in response to a challenge to the rumen environment, which was provided by administering an acid load, and 2) to determine whether the nature of the food choice offered to sheep has an effect upon the response made. As a corollary, a study was made of the food intake and diet selections of the sheep after introduction to a new food or food choice to determine how they respond to a food-mediated challenge to the rumen environment. Furthermore, the patterns of food intake and diet selection were measured over a 24-h period on day 9 of each experimental period.

Days 1-7 of each 21-day period were allowed for adaptation to the new food or food choice offered. During days 15-17 the sheep received a rumen infusion over a 4-h period (Infusion, 1000-1400 h), which was administered via a piece of tubing, inserted through the rumen cannula, according to the procedure described by Cooper et al. (1995a). The infusate was a 1 M solution of HCl (400 mM; 795 mOs/kg). In a previous experiment (Cooper et al. 1995a), it was shown that rumen pH declined by 0.5 units when such a solution was infused, relative to the saline controls. It was presumed that the administration of the chosen solution would also increase rumen osmolality.
Management and Measurements

During the experiment food refusals were removed at 0800 h each day, weighed and then discarded, after which fresh food and water was offered. The sheep had free and continuous access to water, throughout the experiment.

Training period

At the start of the experiment all sheep received a 10-day training period, in which the sheep had the opportunity to gain experience of the foods to be offered subsequently. During this period each sheep was offered free and continuous access to two foods in an alternating pattern according to a modification of the procedure used by Kyriazakis & Oldham (1993). The foods each sheep received was determined by the square to which it had been allocated.

Incidence of rumen sampling and measurements of food and water intake

During days 1-7 of each period a rumen sample was taken at 1200 h (see below). From experience of earlier work (Cooper et al. 1995a), a point sample at this time showed least variation in rumen pH for single foods, and so was considered to be sufficient to indicate how the rumen environment (pH and osmolality) alters with time in response to a change of food/food choice. On day 9 the food intake of the sheep was measured manually every two hours over a 24-h period, starting at 0800 h.

Food intake was recorded at 2-h intervals (0800-1600 h) on days 12-13, days 16-17 (when the infusion was administered) and days 20-21; measurements of daily water intake were also made on these days. On days 13, 17, and 21 rumen samples were also taken every two hours (0800-1600 h). Rumen sampling and food intake recording continued after the infusion had ceased as we have previously shown (Cooper et al. 1995a) that the effect of an acid rumen infusate on rumen pH is still present two hours after the end of the infusion.
Rumen sampling and sample processing

Efforts were made to ensure that the c 80 ml samples of rumen liquor were from the same parts of the reticulo-rumen. The pH of the strained rumen liquor samples was measured immediately using a glass electrode. A sample of strained rumen liquor was centrifuged at 1500 rpm for 20 minutes; the supernatant was filtered using 0.45 μm microbial filters (Whatman Ltd., Maidstone, England) and its osmolality was measured using a Freezing point Osmometer (Knauer, Holstegger, Germany).

Statistical Analysis

All data from the experiment were analysed using GENSTAT 5.3 (Lawes Agricultural Trust, 1993). Between-squares comparisons were made using data from the single food and food choice treatments; these comparisons were made using a Residual Maximum Likelihood procedure (REML; Patterson & Thompson, 1971). The diet selection data were expressed as either the proportion of the high ED food selected, food B (Squares 1 & 2), or the proportion of the pelleted low ED food (Food P) selected (Square 3).

Days 1-7 of each period were considered as an adaptation sub-period. The daily food intake and diet selection data from this sub-period and the daily measurements of rumen pH and osmolality were analysed by fitting the fixed effects of feeding treatment and day. The food intake and diet selection data from the continuous recording period of day 9 were analysed by fitting feeding treatment and time as fixed effects. The analysis of daily food intake and diet selection data from the Control and Infusion sub-periods, days 11-13 (Control 1), days 15-17 (Infusion) and days 19-21 (Control 2) used feeding treatment, experimental sub-period and day as fixed effects.

The same fixed effects were used in the more detailed analysis of food intake and diet selection data from days 12-13, days 16-17 and days 20-21. These data were divided into the following
time intervals (0800-1000 h, 1000-1200 h, 1200-1400 h, 1400-1600 h and 1600-0800 h),
which were treated separately. Rumen pH and osmolality data from days 13, 17 and 21 were
divided into the five sampling times, which were treated separately. The fixed effects of
feeding treatment and experimental sub-period were fitted in the analysis of these data. In all
analyses all two-way interactions between the fixed factors were fitted. Wald tests (Lingren,
1976) were used to test the significance of the fixed effects within each model; comparisons
between treatment means were made using t-tests.

RESULTS

The results are presented separately as measurements taken during the adaptation sub-period
of each Latin square period (days 1-7), the 24-h continuous recording sub-period (day 9), the
days when a rumen infusate was administered (Infusion: days 15-17) and when no infusate
was administered (Control 1: days 11-13 & Control 2: days 19-21). This was considered to be
the most appropriate means of addressing the hypotheses under test. The diet selections made
by the sheep offered food choices have been expressed as either the proportion of high ED
food selected (Food B, B/R and B/P) or the proportion of the pelleted low ED food selected
(food P, P/R). There was a significant effect of sheep and period on all measurements taken
during the whole experiment.

Rumen pH, rumen osmolality, food intake and diet selection during the adaptation sub-
period.

The effects of feeding treatment on daily measurements of rumen pH (Daily pH), rumen
osmolality (Daily Os), daily food intake (DFI) and diet selection are given in Table 5.2. There
was no significant effect of day on daily pH, daily Os or DFI, and therefore the results are
presented as means taken across days. Daily pH on food R was higher (P<0.05) than on food
P, which in turn was higher (P<0.05) than daily pH on food B. Daily Os followed the opposite
pattern, as it was lower on food R than on foods P and B; daily Os on the latter two foods did not differ. On the food choices P/R and B/R daily pH was higher (P<0.05) than on the food choice B/P. There were no significant difference in the daily Os on the three food choices.

On the single food treatments daily food intake was not significantly different. DFI was higher on the food choice treatments P/R and B/R (P<0.05) than on the food choice B/P. The proportion of food P or food B selected on the food choice treatments P/R, B/R and B/P increased after day 1, but did not vary subsequently: 377, 674, 691, 673, 696, 695 & 716 g food B or P/kg DFI (sed 49.2; p<0.001, for days 1-7 inclusive, across the three food treatments). The proportion of food B selected on the food choices B/P and B/R was lower (P<0.05) than the proportion of food P selected on the food choice P/R.
Table 2: Rumen liquor pH (Daily pH), osmolality (Daily Os, Os/kg) daily food intake (DFI, g/d) and diet selection (DS, proportion of food B or food P, g/kg DFI) during days 1-7 (adaptation sub-period) of each period. Rumen liquor samples were taken at 1200 h.

<table>
<thead>
<tr>
<th>Single foods</th>
<th>Food choices</th>
<th>SED</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>P</td>
<td>B</td>
<td>B/R</td>
</tr>
<tr>
<td>Daily pH</td>
<td>6.50a</td>
<td>5.99b</td>
<td>5.59c</td>
</tr>
<tr>
<td>Daily Os</td>
<td>321a</td>
<td>397b</td>
<td>390b</td>
</tr>
<tr>
<td>(Os/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFI (g/d)</td>
<td>1525ab</td>
<td>1781ab</td>
<td>1500ab</td>
</tr>
<tr>
<td>DS (g/kg) DFI</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SED, standard error of difference; NS, significant; *, P<0.05, ***, P<0.001. a, b, c, Means with differenet super scripts differ by P<0.005.
Food intake and diet selection during the 24 h continuous recording sub-period.

Sixty-six percent (se 2.4) of daily food intake was consumed during the period of artificial lighting (0700-1900 h). The consumption of food was greatest during two time intervals, 0800-1000 h and 1600-2000 h, within which 34% (se 2.8) of DFI was consumed. The proportion of food B or food P selected during the interval 0700-1900 h was not significantly different from that selected during the interval 1900-0700 h (622 & 670 g/kg food intake (sed 95.8; p>0.05) for intervals 0700-1900 h & 1900-0700 h respectively).

Rumen pH and osmolality during the Infusion and Control sub-periods

The changes of rumen pH with time (0800-1600 h) during the Control and Infusion sub-periods are given in Figures 5:1a (Single foods) and 5:1b (Food choices). Data from the two Control sub-periods were not significantly different, and so they have been combined for the purpose of presentation. At 1200 h and 1400 h, rumen pH during the Infusion sub-period was lower (p<0.001) than during the Control sub-periods. At the cessation of the infusion (1400 h) rumen pH began to return to levels that were comparable with the Control sub-periods. Rumen pH on food R was consistently higher (pc0.05) than on food P, which in turn was higher (p<0.05) than on food B. At all sampling times rumen pH on the food choices P/R and B/R was higher (pc0.05) than on the food choice B/P.

At all sampling times rumen osmolality during the Infusion sub-period did not differ significantly from the Control sub-periods being 372, 367, 380 (Overall means) (sed 9.2 mOs/kg, p>0.05) for Control 1, Infusion and Control 2 respectively. Comparisons of data from each sampling time show that rumen osmolality was lower on food R than on foods P and B: (Overall means) 348, 393 and 400 (sed 23.5 mOs/kg, p<0.05) respectively. Rumen osmolality was not significantly different on the three food choice treatments at any sampling time.
Figure 5:1 Mean rumen liquor pH (0800-1600 h) of rumen fistulated sheep when given 1l rumen infusion of 400 mM HCl (1000-1400 h) or during the Control sub period. Sheep were offered either single foods or food choices. (For details of the foods see Table 5:1).
Daily food and water intake and diet selection during the Control and Infusion sub-periods

The effects of feeding treatment and experimental sub-period on daily food intake, diet selection and daily water intake during the Infusion and Control sub-periods are given in Table 5:3. There was no effect of time of day on DFI or diet selection and so the results have been pooled across time of day.

On all feeding treatments DFI was significantly reduced (p<0.001) during the Infusion sub-period. The most significant reduction in daily food intake was on food B; as a result the DFI on the sheep on food B during the Infusion sub-period was significantly lower (p<0.05) than on foods R and P. During the Infusion and Control sub-periods DFI on the food choice B/P was lower (p<0.05) than on the food choices B/R and B/P. Diet selections made during the Infusion sub-period were not significantly different from the Control sub-periods. The proportion of food P selected on the food choice P/R was significantly higher (p<0.01) than the proportion of food B selected on the food choices B/R and B/P. The sheep offered the food choices B/R and B/P selected similar proportions of food B. Daily water intake on the single foods was not significantly different, nor was there any significant difference in daily food intake on the food choices. During the Infusion sub-period daily water intake was significantly reduced on all feeding treatments.
Table 5.3: Mean daily food intake (DFI, g/d), diet selection (DS, proportion of food B or food P, g/kg DFI) and daily water intake (DWI, g/d) of sheep during the 3-day Infusion sub-period and 3-day Control sub-periods (Control 1 & Control 2).

<table>
<thead>
<tr>
<th>DFI (g/d)</th>
<th>Single foods</th>
<th>Food choices</th>
<th>SED</th>
<th>Significance of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>B</td>
<td>B/R</td>
</tr>
<tr>
<td>Control 1</td>
<td>1942&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1955&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1445&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1998&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Infuse</td>
<td>1702&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1708&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>975&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1828&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control 2</td>
<td>1740&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1908&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1690&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2154&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DS (g/kg DFI)</th>
<th>Single foods</th>
<th>Food choices</th>
<th>SED</th>
<th>Significance of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>530&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Infuse</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>525&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>627&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DWI (g/d)</th>
<th>Single foods</th>
<th>Food choices</th>
<th>SED</th>
<th>Significance of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>3996&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3759&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3236&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4165&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Infuse</td>
<td>2871&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3277&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1822&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3293&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Control 2</td>
<td>4064&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4591&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3346&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3372&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SED, standard error of difference; NS, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

a, b, c, Comparisons are made within rows and means with different superscript differ by P<0.05.
Food intake and diet selection during two hour intervals between 0800-1600 h of the Control and Infusion sub-periods.

The effects of feeding treatment and experimental sub-period on food intake and diet selection at specific time intervals during the Infusion and Control sub-periods are given in Tables 5:4 and 5:5 respectively. The only effect of Infusion sub-period on the diet selection of sheep was during the time interval 1000-1200 h, in which the proportion of food B or P selected by the sheep decreased (p<0.05) relative to the Control sub-periods. There was no significant effect of the experimental sub-period on food intake during this time interval. At the time intervals 1200-1400 h and 1400-1600 h, food intake was reduced on food B and the food choice B/P (p<0.001) during the Infusion sub-period, relative to the Control sub-periods; during the same time intervals food intake on the other feeding treatments was not significantly reduced during the Infusion sub-period; for this reason the interaction between experimental sub-period and feeding treatment was significant.

There was no significant effect of the Infusion sub-period on the dietary choices made by the sheep during the time intervals 1200-1400 h and 1400-1600 h. The proportion of food P selected by the sheep given the food choice P/R was significantly higher than the proportion of food B selected on the food choices B/R and B/P at the time intervals: 1400-1600 h and 1600-0800 h (p<0.01). During the Infusion sub-period, intake of food B was reduced within the time interval (1600-0800 h), relative to the Control sub-periods; however since there was no significant effect of experimental sub-period on food intake on the other feeding treatments, the interaction between experimental period and feeding treatment was significant.
Table 5.4: Food intake (g) of sheep on the single food and food choice treatments (for details of the foods see Table 1) during the Infusion sub-periods (Infuse) and the Control sub-periods (Control 1 and Control 2). Rumen infusate of 450 mM HCl was administered over 1000-1400 h.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Single foods</th>
<th>Food choices</th>
<th>SED</th>
<th>Significance of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>B</td>
<td>B/R</td>
</tr>
<tr>
<td>0800-1000 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>245</td>
<td>275</td>
<td>150</td>
<td>349</td>
</tr>
<tr>
<td>Infuse</td>
<td>221</td>
<td>182</td>
<td>131</td>
<td>203</td>
</tr>
<tr>
<td>Control 2</td>
<td>255</td>
<td>263</td>
<td>208</td>
<td>319</td>
</tr>
<tr>
<td>1000-1200 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>137</td>
<td>169</td>
<td>102</td>
<td>158</td>
</tr>
<tr>
<td>Infuse</td>
<td>190</td>
<td>181</td>
<td>88</td>
<td>158</td>
</tr>
<tr>
<td>Control 2</td>
<td>167</td>
<td>162</td>
<td>124</td>
<td>173</td>
</tr>
<tr>
<td>1200-1400 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>133</td>
<td>119</td>
<td>114</td>
<td>176</td>
</tr>
<tr>
<td>Infuse</td>
<td>122</td>
<td>127</td>
<td>55</td>
<td>127</td>
</tr>
<tr>
<td>Control 2</td>
<td>89</td>
<td>153</td>
<td>152</td>
<td>138</td>
</tr>
<tr>
<td>1400-1600 h</td>
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<td></td>
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<td></td>
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<td>Control 1</td>
<td>201</td>
<td>270</td>
<td>129</td>
<td>188</td>
</tr>
<tr>
<td>Infuse</td>
<td>164</td>
<td>115</td>
<td>67</td>
<td>217</td>
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<tr>
<td>Control 2</td>
<td>154</td>
<td>212</td>
<td>148</td>
<td>272</td>
</tr>
<tr>
<td>1600-0800 h</td>
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<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>1214</td>
<td>1186</td>
<td>945</td>
<td>1257</td>
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<tr>
<td>Infuse</td>
<td>983</td>
<td>1173</td>
<td>509</td>
<td>1113</td>
</tr>
<tr>
<td>Control 2</td>
<td>1058</td>
<td>1218</td>
<td>1051</td>
<td>1256</td>
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</tbody>
</table>

SED: standard error of the difference: *: P<0.05, **< P<0.01; ***: P<0.001, NS: Non significant
Table 5.5: Diet selections of the sheep that were given the food choices B/R, B/P and P/R (for details of the foods see Table 1) during the 3-d Infusion sub-period (Infuse) and the 3-d Control sub-periods (Control 1 & Control 2). The data are presented as the proportion of food B or P selected (g/kg food intake) during specific time intervals. Rumen infusate of 450mM HCl was administered over 1000-1400 h.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>B/R</th>
<th>B/P</th>
<th>P/R</th>
<th>SED</th>
<th>Food choice</th>
<th>Significance of:</th>
<th>Sub-Period</th>
<th>F x SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0800-1000 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>502</td>
<td>657</td>
<td>708</td>
<td>157.7</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Infuse</td>
<td>413</td>
<td>543</td>
<td>450</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>306</td>
<td>725</td>
<td>546</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000-1200 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>550</td>
<td>564</td>
<td>788</td>
<td>144.2</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>Infuse</td>
<td>424</td>
<td>550</td>
<td>565</td>
<td></td>
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<td></td>
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<td>716</td>
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<tr>
<td>Control 1</td>
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<td>513</td>
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SED standard error of the difference; *: P<0.05; **: P<0.01
DISCUSSION

It has been proposed (Cooper et al. 1995a) that sheep are able to alter diet selections in the short-term to promote or maintain rumen conditions that would be conducive to microbial growth and cellulolysis, and which would sustain a level of intake that would allow the animal's requirements for energy and protein to be met. The maintenance of such rumen conditions would be beneficial to the host as the volatile fatty acids that are produced from microbial fermentation provide up to 70% of absorbed energy (Blaxter, 1967); microbial activity is also a vital source of protein (Beever 1993). Maintaining rumen pH and osmolality within physiological limits will also minimise the risk of conditions such as acidosis (Critchlow, 1988), rumen stasis (Lopez et al. 1994) and also damage to the rumen epithelia (Dirksen, 1970).

In response to a direct challenge to the rumen environment sheep, which have been offered a choice between foods of high and low ED, reduce the proportion of the high ED food selected (Engku Azahan & Forbes, 1992; Cooper et al. 1995a). The consumption of a high ED food would be expected to have an adverse effect on the rumen environment (Mould & Ørskov, 1984; Block & Shellenberger, 1980) and therefore the reduced selection of a high ED food under such circumstances is in accordance with the above hypothesis. We have also shown (Cooper et al. 1995b) that sheep appear to select foods which have physico-chemical properties that would be expected to promote the rumen conditions described above. The objective of the current experiment was to test the hypothesis that, if the rumen conditions were directly challenged, sheep would select a diet to minimise further disruption, and that the nature of the food choice offered would be expected to affect the response.
When ruminants are switched from one food to another, a transition period is considered necessary to ensure that the ruminal micro-flora (Mackie et al. 1978), the epithelia of the gastro-intestinal tract (Sakata et al. 1980) and the host animal's metabolism (Nikolic et al. 1980) adapt to cope with a dietary change. Therefore it would be expected that ruminants would change their diet selection and food intake during such a period. In this experiment, however, there was no evidence to indicate that the sheep did alter their feeding behaviour when the food or food choice was changed. On all food choice treatments dietary choices were established by day 2 of the adaptation sub-period, and there were no subsequent changes.

As the dietary choices of the sheep were established within a 2-day period, this suggests that the processes involved in the determination of choice were affected very quickly. It is also probable that the changes in diet selection which occurred were independant of any major changes in either microbial fermentation or the supply of nutrients to the post-ruminal digestive tract, as changes to these processes would be unlikely to occur within such a short time-scale (Mackie et al. 1978; Van Soest, 1982). As there were no significant changes in rumen pH or osmolality during the adaptation sub-period, it is possible that the fermentation patterns of the foods used were of sufficient similarity that no major changes in the nature or dynamics of rumen fermentation occurred between periods. However this begs the question as to what properties of the foods influenced dietary choice and what processes were responsible for the rapid recognition of these qualities, which allowed stable diet selections to be established so quickly.

The food intake and diet selection of the sheep were also studied during a 24-h period of continuous recording. This indicated that the sheep maintained a diurnal pattern of feeding behaviour, whilst in metabolism cages, that was similar to the feeding behaviour of sheep in less controlled environments (Lynch et al. 1992), as the sheep consumed 64 % of daily food
intake during the period of artificial lighting (0700-1900 h). Similar diet selections were made during the periods of light and dark, which indicates that there was no evidence of a diurnal pattern to the dietary choices of the sheep. The absence of a diurnal pattern to dietary choices, made in the present experiment, is at variance with Parsons et al. (1994), who found that grazing sheep offered pasture containing clover and grass ate clover predominantly in the morning but predominantly grass in the late afternoon/early evening. Parsons et al. (1994), however, did not record the behaviour of the sheep during the period of darkness.

The influence of feeding treatment on responses to a challenge to the rumen environment

The main objective of the experiment was to examine whether the dietary choices made by sheep would change in response to a challenge to the rumen environment, which was provided by administering an acid load, and to determine whether the nature of the food choice offered to sheep has an effect upon the response made.

The results obtained from the single food treatments, foods P, R and B, can be used to interpret fully the dietary choices made by the sheep. A similar approach has been recommended by Emmans (1991) and used by Kenney et al. (1984) and Kyriazakis & Oldham (1993). It was expected that food B would have been rapidly fermented in the rumen as it was a starch-based food (67% barley), unlike foods P and R that were substantially based upon cellulose and hemicellulose (84% alfalfa) (Mould & Ørskov, 1984). In addition it was presumed that the buffering capacity of food B was less than the buffering capacity of foods R and P, as alfalfa is known to have a high cation exchange capacity (McBurnley et al. 1983) and a high pectin content (Van Soest et al. 1991), although these properties were not measured. Rumen pH on food B was low relative to contemporary measurements of rumen pH on foods P and R. The most significant reduction in food intake during the Infusion sub-period was also most evident on food B, which implies that the sheep on food B were less able to
attempt to maintain food intake when the rumen environment was challenged by the acid infusate.

It is likely that the physical form of the alfalfa foods would also have had an effect on the rumen environment and, indeed, rumen pH on food P was lower than on the long-chop alfalfa, food R, and rumen osmolality on the latter was lower than on food P. These results are consistent with Marshall et al. (1992) and Teller et al. (1989) and suggest that the buffering capacity of alfalfa was reduced when it was physically processed. Physical processing of forages, to produce foods such as food P, is associated with reduced levels of mastication and salivation (Campbell et al. 1992; Block & Shellenberger, 1980). However the effect of pelleting the alfalfa to produce food P did not eliminate its buffering capacity entirely, as rumen pH on food P was higher than on food B.

In general, more food B was selected when it was paired with food R than when it was paired with food P but the effect of the physical form on the dietary choices of the sheep was less evident in the current experiment than in the previous study (Cooper et al. 1995b). Although differences in the potential intake rate on foods R and P could affect dietary choice (Kenney et al. 1984), it has already been suggested (Cooper et al. 1995b) that other attributes of foods R and P (for example buffering capacity) may also affect dietary choice. It is possible that a property of the alfalfa which was present in relative abundance in the unprocessed food R was diluted by the grinding and pelleting processes which were used to create food P. A higher amount of food P may have been needed in the diet selected by sheep to produce the same effect as a unit amount of food R. The proposed difference between foods P and R would be of greatest importance when the balance within the rumen was challenged.

The dietary choices made by the sheep on the B/R and B/P food choices within the first 2-h interval (1000-1200 h) of the rumen infusion indicate that they responded to the marked
decline in rumen pH by reducing the proportion of food B or food P selected such that there was a decreased intake of food B or food P respectively. The quantity of food B was also reduced on the food choice B/P, however this was mainly due to a general reduction in food intake with little change in the proportion of B selected. The diet selections of the sheep on the food choices B/R and P/R during this interval are consistent with the proposition that sheep respond to induced disturbances to the rumen environment by reducing the intake of foods (Foods P and B) that would be expected to have a considerable impact on the rumen environment. This dietary strategy would accord with results from other experiments (Engku Azahan & Forbes, 1992; Cooper et al. 1995a).

In the second 2-h interval of the rumen infusion (1200-1400 h) the sheep did not make further alterations in their dietary choices in response to the continued depression of rumen pH. Instead, food intake declined further on all food choice treatments; this was most marked on the food choice B/P. These results indicate that the ability of sheep to minimise further disruptions of the rumen environment, by altering their dietary choice, can only occur if specific attributes of the rumen environment, such as rumen pH and osmolality, remain within certain limits. Once these limits have been exceeded, the only dietary strategy available to sheep, to minimise further disruptions of the rumen environment, is to reduce food intake. As the basal rumen pH on the food choice B/P was lower than on the food choices B/R and P/R, it is possible that rumen conditions already lay outside the hypothetical limits mentioned above. As a consequence, the sheep on the food choice B/P were only able to respond to the imposed challenge to the rumen environment by reducing their food intake.

It is possible that the first attempt that could be made by sheep to restore conditions within the rumen, would be to reduce food intake. Such a drastic response to a challenge to the rumen environment is the only strategy available to sheep given access a single food, and it may be associated with some benefits, such as an increase in rumen pH. From the observations made
in the present experiment we suggest that sheep offered a choice of foods can follow an alternative strategy, in which that the first response of sheep to an imposed challenge to the rumen environment is to alter their diet selection, if this is possible, to minimise further disruption to the rumen environment. If rumen conditions remain disrupted, the next response of sheep is to reduce food intake.

This hypothesis is consistent with the results of Kyriazakis & Oldham (1993), who have shown that sheep offered a food choice which included a food loaded with urea (5 % w/w) appeared to attempt to avoid a high level of ruminal ammonia, by altering their dietary choices, rather than by reducing their total food intake. Parallels can be drawn between this hypothetical system which may operate to maintain balance within the rumen and the various systems that operate to maintain the body's homeostasis, such as the regulation of plasma glucose levels, as there are primary and secondary responses to counteract a change in plasma glucose concentration (Guyton, 1986).

*Long term consequences of the rumen infusion*

The effect of the rumen infusion on food intake persisted after the cessation of the infusion; this was most evident when food B was offered alone. However the effect of the administration of an intra ruminal infusate on dietary choice did not remain after the cessation of the treatment. These results accord with Engku Azahan & Forbes (1992) and Cooper et al. (1995a) who also showed that the effect of administering an intra-ruminal solution on dietary choice does not linger. In contrast, the dietary choices of sheep are altered for longer periods when LiCl is used to produce conditioned aversions to foods (Burritt & Provenza, 1991). Such changes in dietary choice can last for 60 days (Thorhallsdottir et al. 1987). Quite different regulatory systems must therefore be used to distinguish between the short-term tactical challenge that is elicited by agents such as HCl and the strategic aversions generated from the use of emetics such as LiCl.
Effects of rumen pH and osmolality

In a previous study (Cooper et al. 1995a) sheep which were offered a choice between a high and low ED food, reduced their intake of the high ED food between 1000-1600 h in response to the administration of acid, alkali and saline rumen infusions between 1000-1400 h. Those dietary choices (Cooper et al. 1995a) were consistent with the proposal that the sheep changed their diet selections in an attempt to minimise further increases in rumen osmolality rather than pH. However in the current experiment it is probable that the sheep altered their dietary choices to minimise further changes in rumen pH, rather than to minimise further changes in rumen osmolality. There was no evidence that rumen osmolality was affected by the administration of the rumen infusate, nor did the sheep increase their water intake during the Infusion period.

Different time scales were used in these two experiments to express the dietary choices made. In the earlier experiment (Cooper et al. 1995a) the dietary choices of the sheep were expressed using data collected over a six-hour period (1000-1600 h). Data collected under similar circumstances in the current experiment were divided into 2-h intervals. The apparent effect of rumen osmolality on dietary choice remained, but did not reach significance when the data from the earlier experiment (Cooper et al. 1995a) were also divided into 2-h intervals for the purpose of analysis; this indicates that differences in the statistical models used in these two experiments do not account for the inconsistent effect of rumen pH on dietary choice.

It is possible that the different physico-chemical properties of the foods used in the two experiments may account for the inconsistent effect of rumen pH on dietary choice. The consumption of pelleted foods, such as foods B and P, and the foods in the previous experiment (Cooper et al. 1995b) may be expected to reduce the production of the primary buffering agent of the rumen, saliva (Carter & Grovum, 1990a), thereby reducing the buffering capacity of the rumen. However the consumption of a long-chop forage, such as
food R, would be expected to stimulate the production of saliva (Block & Shellenberger, 1980). When the acid rumen infusate was administered in the current experiment, it is presumed that the sheep on the food choices B/R and P/R reduced their intake of the pelleted foods B and P respectively whilst maintaining their intake of food R to ensure the buffering capacity of the rumen did not decline further. It is proposed that the pelleted form of both of the foods used in the previous experiment (Cooper et al. 1995a) precluded the sheep from attempting to maintain the buffering capacity of the rumen by altering their dietary choice. The apparent effect of rumen osmolality on dietary choice which had been reported (Cooper et al. 1995a) may stem from an inability to cope with changes in rumen pH due the nature of the food choice offered.

**Conclusions**

The results of this experiment are consistent with the proposal that the nature of the food choice offered to the sheep has an effect upon their ability to respond to a direct challenge to the rumen environment. Where the challenge to the rumen environment was such that a shift in food properties (diet choice) could re-equilibrate the rumen environment within an 'acceptable' range of physico-chemical characteristics, the primary response was in diet choice. If such a re-equilibriation could not readily be achieved, the sheep's response was to reduce food intake. The sheep responded to the administration of the acid rumen infusate by reducing the intake of foods that would be rapidly fermented and which would not stimulate salivation. In particular it appears that the sheep responded to changes in rumen pH by making short-term changes in their dietary choice, rather than making more permanent changes in diet selection.
Chapter Six

General Discussion
The purpose of the five experiments described in the previous four chapters has been to consider:

1. The effect of physiological state on the diet selection of sheep.
2. The effect of the rumen environment on the dietary choices of sheep.

The general themes that will be considered in this chapter are stated below:

1. The effect of physiological state and dietary constraints on diet selection.
2. The effect of the rumen environment on diet selection.
3. The nature of the response that is made when the rumen environment is disrupted.

THE EFFECT OF PHYSIOLOGICAL STATE ON DIET SELECTION

Some monogastric examples

The first objective of this thesis was to study the effect of physiological state on the diet selection of sheep. It is known that monogastric animals can select a diet that appears to allow them to meet the nutritional requirements of their physiological state. For example female pigs that are offered a choice between foods of high and low crude protein (CP) concentration select a diet which has a lower CP content than the diet chosen by contemporary entire male pigs (Kyriazakis et al. 1990); such a dietary choice would be consistent with the lesser capacity for growth and protein deposition of female animals. During pregnancy and lactation the protein content of the diet selected by rats increases as the requirements of the animal increase (Richter, 1938; Leshner, 1972). It is accepted that laying birds are also able to select a diet which has protein (Emmans, 1977) and calcium (Classen & Scott, 1982) concentrations that appear to be appropriate to their level of egg production. The previous nutritional status of monogastric animals also affects diet selection, as pigs which have been made either 'fat' or 'thin' by dietary means have been shown to select diets which allowed the two groups to
'correct' their body composition toward a common level of body fatness (Kyriazakis & Emmans, 1991).

The effect of physiological state on the diet selection of ruminants

The suborder Ruminantia; family Bovidae (which includes from the domestic species, sheep, goats and cattle) are widely distributed throughout the world (Stevens, 1988). In these diverse environments they encounter foods which vary in their distribution, nutrient composition and digestibility (Illius & Gordon, 1990) and from these they must select a diet that enables them to grow and reproduce successfully. As it is known that ruminants will alter their food intake in an attempt to ensure that the different nutrient requirements of their various physiological states are met (Weston, 1982), it is therefore logical to consider whether ruminants also have the capacity to make changes in their diet selections for this purpose.

There is a relative paucity of experiments which have used the ruminant as a model to study the effect of physiological state on diet selection (Gordon & Tribe, 1951; Hou, 1991; Parsons et al. 1994; Newman et al. 1994; Kyriazakis et al. 1994). A number of factors can be cited which account for this limited number. The body composition of monogastric animals can be readily manipulated by nutrition (Campbell et al. 1983), but it is more difficult to manipulate the body composition of ruminants (Bass et al. 1990) unless use is made of either very controlled feeding regimes (Ørskov et al. 1971) or anabolic agents (bST: Johnson et al. 1987; β-agonists: Buttery & Dawson, 1987). Furthermore, unless exogenous hormones are used, the opportunities to study the effect of specific physiological states (pregnancy and lactation) is limited to certain seasons.

There are, however, a few cases where the effect of physiological state on diet selection of ruminants (sheep) has been studied (the examples cited above). Kyriazakis et al. (1994) have demonstrated that the presence of a parasitic burden appears to cause sheep to alter their
dietary choices, as sheep which have received a daily subclinical dose of *Trichostrongylus colubriformis* increased their selection of a high-protein food. It was proposed that this adjustment in dietary choice was to overcome the impairment in N metabolism that is associated with a *T. colubriformis* infestation (Sykes & Coop, 1976). On the other hand, Hou (1991) reported that the level of body fatness had no effect on the dietary choices made by sheep which were offered foods of different CP concentration. It is possible that limiting metabolic constraints, such as the ability of the liver and kidneys to deaminate and dispose of excess amino acids, prevented the two groups of sheep from selecting diets that would restore their body fatness to a common level. Furthermore, as the number of sheep available to Hou (1991) was limited, it is also possible that an effect of body fatness on diet selection could not be detected statistically.

In this thesis an indirect comparison can be made between the dietary choices made by young, growing sheep (10 weeks at the start; Chapter 4) and more mature sheep (>21 months at the start; Chapter 5). Both groups of sheep were offered food choices which consisted of a low energy-dense (ED) food based on alfalfa and a high-ED food based on barley (B). The young, growing sheep selected a diet that contained a higher proportion of the high ED food than the more mature sheep; the dietary choices of the two groups were: 679 (se 65.2) and 586 (se 54.7) g food B/ kg daily food intake (DFI) respectively. Young, growing sheep would be expected to have a lesser ability to digest fibrous foods (Liebholz, 1991) and to have a smaller rumen capacity (Milne, 1991) relative to more mature sheep. These physiological differences may account for the tendency for the younger sheep to show a greater preference for the high ED food (food B). In addition, young, growing sheep have a greater energy requirement per unit metabolic body weight (Weston et al. 1989); this could also account for the higher proportion of food B selected by the younger sheep studied in Chapter 4. In contrast, the results of Hou (1991) indicate that there is no effect of age on the selections made by sheep between foods of different CP content. The lambs studied by Hou (1991) were more than three
months of age at the beginning of the experiment, and, therefore, they may have been too 'old' to require a diet with a protein content that was vastly higher than the more mature animals (Agricultural & Food Research Council, 1992); this possibility was also acknowledged by Hou (1991).

In monogastric animals the states of pregnancy and lactation (Richter, 1938; Leshner, 1972), have been used as a useful means of 'manipulating' the nutrient requirements of animals to study the effect of physiological state on diet selection, as an animal's nutrient requirements increase markedly during pregnancy and lactation. Amongst ruminants the burden that is placed upon pregnant and lactating animals is considerable (Agricultural Research Council, 1980; AFRC, 1992); in sheep specifically, requirements for protein and energy increase rapidly over the last two months of pregnancy in order to supply the growing needs of maternal tissues and the foetus itself, which gains 80 % of its birth weight during this interval (Robinson, 1977). The objective of the work reported in Chapter 2 was therefore to examine whether sheep alter their dietary choices to meet the enhanced physiological needs which are imposed by late pregnancy. The effect of pregnancy of the dietary choice of sheep was previously studied by Gordon & Tribe (1951), where few of the ewes successfully lambed, and it was concluded that sheep are not able to show 'nutritional wisdom'. It is likely that the foods that were available to the sheep studied by Gordon & Tribe (1951) did not permit these animals to select a diet that was appropriate to their requirements. (A more detailed criticism of this experiment has already been made in Chapter 2).

In Chapter 2 pregnant and contemporary non-pregnant ewes were offered choices between foods of different CP concentration to determine whether late pregnancy has an effect upon the dietary choice of sheep. Two food choices were used, one was of a high ED and the second was of a low ED. As the high-ED foods included a high proportion of starchy materials, it was expected that these foods would yield a high proportion of propionate when fermented in
the rumen relative to the low ED foods (Sutton et al. 1986). The objective of attempting to manipulate the level of propionate production in the rumen was so that it would be possible to consider whether the selection for protein by ewes in late pregnancy is affected by the availability of non-amino acid glucogenic precursors, such as propionate. When the food choice offered was of a high ED, the pregnant ewes selected a higher proportion of the food with a high CP content than the non-pregnant ewes. These dietary choices were consistent with the hypothesis that sheep can exhibit state-dependant diet selection. It was not possible to determine whether the attempts to manipulate ruminal fermentation achieved the intended aims, and so it is not known whether the availability of non-amino acid glucogenic precursors prompted the sheep to alter their diet selection.

Dietary constraints and physiological state: the interaction between these and the effect on diet selection

The similarity of the dietary choices made by the ewes made by the ewes on the low ED food choice in Chapter 2 indicates that these animals were not making dietary choices according to the nutrient requirements of their physiological state, instead it is suggested that other priorities were motivating their diet selection which were common to both groups. It is suggested that the synchronous supply of protein and energy resources to the rumen was such a priority. A balance between the ratio of supply of energy and protein ensures rapid microbial growth (Sinclair et al. 1993) as well as facilitating a high level of food intake (AFRC, 1992). Pregnant and non-pregnant ewes on the low-ED food choices selected diets which would be expected to have very similar rates of supply of energy and protein (as judged by the effective degradable protein (eRDP)/ fermentable metabolisable energy (fME) ratio: AFRC, 1992) and which were within the range identified by AFRC (1992) as being optimal for microbial protein production.
Although an asynchronous supply of energy and protein to the rumen would restrict microbial protein production (Hespell & Bryant, 1979), it is unlikely that sheep select for 'optimal microbial growth' per se. It is more probable that the processes of dietary choice may be influenced by other consequences of an asynchronous supply of protein and energy to the rumen, namely low rumen pH (Counette et al. 1979), low osmolality (Carter & Grovum, 1900a) and elevated ammonia levels (Oldham et al. 1977). The possibility that the nature of the rumen environment may influence dietary choice has been alluded to by Parsons et al. (1994), who speculated that sheep grazing on a grass/clover sward may choose to include some grass in the diet selected, although this would lower the rate of energy intake, as this would enable a diverse gut microflora to be sustained. The diet selections made by the sheep studied by Parsons et al. (1994) and the sheep offered the low-ED food choice in Chapter 2 are consistent with the proposal that dietary constraints may supercede the priorities of the physiological state, and that rumen conditions may influence the dietary choices made by sheep.

THE EFFECT OF THE RUMEN ENVIRONMENT ON THE DIET SELECTION OF SHEEP.

The second objective of this thesis was to study the effect of the rumen environment on dietary choices. The reticulo-rumen and the microbial activity therein is crucial to the metabolism of the host ruminant (Theodorou & France, 1993). Up to 70% of the energy absorbed by ruminants is derived from volatile fatty acids which are the end-products of microbial fermentation (Blaxter, 1967; Stevens, 1988); the protein synthesised by rumen microorganisms is essential to the nitrogen balance of the host (Asplund, 1986; Beever, 1993). Considerable evidence exists which indicates that rumen conditions can influence the intake of single foods by ruminants, for example rumen pH (Williams et al. 1987), rumen osmolality

Cropper (1987) speculated that a sheep will select a diet that 'enables its rumen to remain in adaptive state'. This suggestion was made to account for the dietary choices made by sheep which selected a mixture of the two foods offered, which were of high and low digestibility, rather than just choosing the food with the higher digestibility. The mixed diet chosen by the sheep in this experiment differed from the diet it would be expected sheep would select when offered such choices, according to the expectation that animals seek to maximise their rate of energy intake (Krebs & McCleery, 1984). However the experiment described by Cropper (1987) has several limitations, and the author alludes to some of these, for instance several of the foods of low digestibility that were used by Cropper (1987) did not limit growth to the extent that they were intended to; this was because unavoidable compromises had to be made in the formulation of these foods. It can be predicted that the foods used by Cropper (1987) would have had different rates of supply of protein and energy to the rumen. This may have had a bearing on the results obtained, as it has been suggested earlier that the objective of avoiding the consequences of an imbalanced supply of energy and protein to the rumen may influence dietary choice. Furthermore, as the number of animals available to Cropper (1987) was small, this may have limited the power of the statistical analyses.

The effect of energy density on the dietary choices of sheep

Before turning to a direct study of the effect of the rumen environment on dietary choice, it was felt necessary to give further consideration to the effect of energy density on dietary choice, given the limitations of the work carried out by Cropper (1987). This was the objective of Chapter 3 (Experiment 1). Three foods of different ED (L (low), H (high) and M (medium,
3/4 L: 1/4 H), but with similar ratios of metabolisable protein: metabolisable energy were offered to young, growing sheep as single foods or as food choices (L/M, L/H and M/H). The sheep offered food H alone grew more rapidly than those offered the foods of lower ED singly; despite this, the sheep on the food choices L/H and M/H did not solely select food H, but instead chose a mixture of both foods offered in a non-random and consistent fashion. These dietary choices were consistent with the results reported by Cropper (1987) and, as more sheep could be used in Chapter 3 (Experiment 1), the relationship between the energy density of the foods offered and the diet selected was more distinct than was reported by Cropper (1987). The dietary choices made by other sheep studied in this thesis (Chapter 3 (Experiment 2); Chapter 4; Chapter 5) and by other groups (eg. Broom & Arnold, 1986) also accord with the hypothesis that the objective of dietary choice in ruminants is not solely to maximise the rate of energy intake.

*Optimising the benefits to be gained from selecting a high ED food*

A number of hypotheses have been advanced to explain why ruminants and other large generalist herbivores select diets that consist of a mixture of the foods available, for example animals may show a preference for rarity (Newman *et al.* 1992); the existence of sampling behaviour has also used to account for mixed diets (Westoby, 1974). Several of these hypotheses have been considered in Chapter 3. It is not felt, however, that any of these hypotheses can entirely account for the feeding behaviour of the sheep in Chapter 3 (Experiment 1).

The dietary choices made by the sheep in Chapter 3 (Experiment 1) can be expressed as proportions of foods L and H, since food M was a mixture of these two foods. This showed that the diet selected by the sheep offered the L/H and M/H food choices contained very similar proportions of food H (680 vs 695 g H/kg DFI, sed 54.2). The similarity of the diet
selections made by the sheep on these food choices and the fact that the animals did not select food H alone probably indicates that there are costs associated with the consumption of a high ED food as well as benefits (high growth rate and low food intake). Measurements of rumen pH and osmolality that were taken in Chapter 3 (Experiment 2) and Chapter 5 show that the costs of solely selecting a high ED food include an adverse effect on the rumen environment (low pH and high osmolality). The creation of such a rumen environment would be associated with reduced cellulolysis (Mould & Ørskov, 1984) and reduced microbial protein production (Hespell & Bryant, 1979; Russel & Strobel, 1993), as well as having an adverse effect on the animal's acid-base balance (Abu Damir et al. 1990), and the integrity of the rumen wall (Dirksen, 1970; Lopez et al. 1994). The proposal that the process of diet selection in ruminants is influenced by attempts to maximise the rate of energy and protein intake, whilst also minimising the associated costs, is consistent with the earlier hypothesis, namely that one of the objectives of dietary choice in ruminants is to maintain rumen conditions within certain physiological limits.

MANIPULATION OF THE RUMEN ENVIRONMENT: THE EFFECT ON THE DIETARY CHOICE OF SHEEP

The microbial processes of cellulolysis and microbial protein production are crucial to the energy and nitrogen balance of the host ruminant (Stevens, 1988). It is not probable, however, that sheep modify their dietary choices, with the specific intent of maximising the rate of these microbial processes. It is more likely that ruminants (sheep) will modify their dietary choices to correct 'imbalances' in the rumen environment such as low rumen pH, high rumen osmolality and high levels of ammonia, as this would enable them to minimise the risk of developing conditions such as acidosis (Critchlow, 1988). Following such a diet strategy would also bring indirect benefits, namely high rates of cellulolysis (Mould & Ørskov, 1984).
and microbial growth (Hespell & Bryant, 1979). To test this hypothesis, it was necessary to manipulate the rumen environment to determine whether sheep will modify their dietary choices to 'correct' any imbalances in the rumen environment. Imbalances in the rumen environment can be created by dietary means, through the use of foods with different physico-chemical properties (Chapter 4 & Chapter 5) or by a more direct means of intervention, namely rumen infusions of materials chosen specifically to alter attributes of the rumen environment, such as rumen pH and osmolality (Chapter 3 (Experiment 2) & Chapter 5).

Using foods to manipulate conditions within the rumen

In Chapter 4 sheep were offered a choice between foods of high and low ED. The high ED foods varied in two dimensions, the level of inclusion of the mineral buffer, sodium bicarbonate (bicarbonate: 0, 1, 2 and 4 % w/w) and the carbohydrate source (barley-based or sugarbeet pulp (SBF)/ barley-based). The low-ED foods varied in one dimension i.e. physical form (alfalfa, long chop or ground and pelleted). It was expected that the manipulation of these physico-chemical properties of foods would have an effect upon the rumen environment (carbohydrate: Van Soest et al. (1991); bicarbonate: Erdman (1988) and physical form: Campbell et al. (1992)). The effect of physical form on dietary selection was also studied in Chapter 5. In this experiment the rumen environment was also manipulated directly through the use of an acid rumen infusate.

The effect of bicarbonate inclusion on dietary choice:

Food intake is increased when bicarbonate is added to readily fermentable foods, which would otherwise be associated with a low rumen pH (Ha et al. 1983; Erdman, 1988). Given this, it was expected that the inclusion of bicarbonate in high ED foods would have the effect of permitting the sheep to chose more of the high-ED food when offered as a choice with a low ED food, as the presence of the dietary bicarbonate would be expected to offset some of the
costs associated with the consumption of the high-ED food. In addition, it was expected that sheep, which were offered a choice between a high-ED food that was supplemented with bicarbonate and an equivalent unsupplemented one, would alter their dietary choices so that there would be a constant level of bicarbonate in the selected diet. However these predictions were not borne out by the results, as the selections made by the sheep offered choices between high and low ED foods were similar irrespective of whether the level of bicarbonate in the high ED food was 0 or 4% (w/w). Furthermore, the level of bicarbonate inclusion (1, 2 & 4 % (w/w) did not appear to affect the diet selections made by the sheep offered a choice between a supplemented food and an equivalent unsupplemented food.

There are two alternative explanations for the non-significant effect of bicarbonate on the dietary choices made by the sheep in Experiment 4. The first is that the sheep were unable to distinguish between the levels of bicarbonate used, because the experiences gained by the sheep during the training period were not appropriate to enable them to discriminate between the three levels of bicarbonate used. Sheep have the ability to learn about the post-ingestive consequences of eating foods which are paired with the emetic substance, LiCl (Burritt & Provenza, 1989; duToit et al. 1991); it appears that they are able to retain and act upon such knowledge for a period of time (up to 60 days) after the initial exposure (Thorhallsdottir et al. 1987). However it is possible that the interval between the first and second exposures needed to be shorter to retain knowledge about foods that elicit post-ingestive consequences that do not lead to actual aversion or do not stimulate the emetic system. Therefore, it is feasible that the interval between the the first (training period) and second (experiment) exposures to bicarbonate in Chapter 4 was too great, in many instances, for the sheep to retain and act upon the experiences gained during the training period.

The second explanation is that the mode of administration of the bicarbonate determined whether it had an effect upon dietary choice. F. D. Provenza (1994; personal communication)
has shown that there is an effect of bicarbonate on the diet selection of sheep when the material is sprayed onto the surface of wheat grains. In contrast, the bicarbonate was incorporated into the food pellets in Chapter 4. Although it is accepted that organoleptic stimuli can have an influence the process of diet selection (Arnold, 1964; Gherhardi et al. 1991), it is unlikely that the effect of a material, such as bicarbonate, would be wholly mediated by its ability to stimulate receptors in the buccal cavity and associated areas, as bicarbonate can also exert potent effects upon other sections of the gastro-intestinal tract (Erdman, 1988). If the opportunity were available to re-examine the effect of bicarbonate inclusion on dietary choice, it would be advisable to ensure that the interval between the training period and second exposure to the bicarbonate is shorter in all instances than could be achieved in Chapter 4. In addition, it recommended that the sheep have experience of all the levels of bicarbonate inclusion to be used prior to the start of the experiment.

The effect of dietary carbohydrate source

It was found that the carbohydrate source used in the high-ED food had an effect on the dietary choices made by the sheep in Experiment 4. The sheep offered the high-ED food based on predominantly barley (639 g/kg food) selected a higher proportion of the low-ED food than those offered a high-ED food based on an SBF/barley mix (322 g SBF & 322 g barley/kg food). The nature of the dietary carbohydrate source affects both the level of ruminal lactic acid (Van Soest et al. 1991) and the buffering capacity of the rumen (McBurnley et al. 1983). Foods containing mainly barley are associated with a lower rumen pH (Rymer & Armstrong, 1989) than foods that contain substantial amounts of SBF. This is due to the lower buffering capacity of barley relative to SBF (McBurnley et al. 1983). In addition the ruminal fermentation of barley can produce considerable amounts of lactic acid. This acid is not produced when SBF is fermented (Van Soest et al. 1991). High levels of ruminal lactic acid can have deleterious effects on food intake (Forbes & Barrio, 1992) and the rumen wall (Dirksen, 1970; Smith et al. 1979).
There do not appear to be any other diet selection experiments in the literature which have studied the effect of dietary carbohydrate source on the diet selections made by sheep. However Rymer & Armstrong (1989) and Williams et al. (1987) have shown that sheep offered single foods containing substantial amounts of SBF are able to consume more than those offered foods in which barley has replaced the SBF. This effect of dietary carbohydrate source on the intake of single foods was quite marked in Chapter 4 and it was suggested that the intake of the sheep offered barley-based food was limited by physiological factors. It is presumed that the sheep which were offered the barley-based food attempted to compensate for the lower buffering capacity of the cereal by selecting a higher proportion of the low-ED food than was chosen by those sheep which were offered the SBF/barley food; such a dietary choice would also have the effect of 'diluting' the lactic acid produced, although this latter possibility is only a speculation as no measurements of ruminal lactic acid level were made in Chapter 4.

The effect of physical form on the dietary choice of sheep

Kenney et al. (1984) have suggested that the effect of the physical form on diet selections could be solely attributed to differences in potential intake rate, as they showed that sheep, offered a choice between dried forages of different chop length (10 mm vs 40 mm), select the forage with the shorter chop length, as this would permit them to maximise their potential intake rate. However the dietary choices made by the sheep in Chapters 4 and 5 indicate that attributes of foods other than potential intake rate may also be of importance. Processed forages have a low capacity to stimulate chewing behaviour (Oltjen et al. 1965), as a consequence these foods have a lower capacity to elicit the production of saliva (Block & Shellenberger, 1980). The production of saliva is of significance as it is the primary buffering agent of the rumen (Turner & Hodgett, 1955, Carter & Grovum, 1990a). The proposal that differences in the extent to which a food stimulated the production of saliva would affect dietary choice was strengthened by the results produced in Chapter 5. Sheep in Chapter 5 that
were offered a choice between a high-ED food (B) and a long-chop alfalfa food (R) had a higher basal rumen pH than those an equivalent choice in which the alfalfa had been ground and pelleted (P) The sheep on choice B/R were able to respond to a direct challenge to the rumen environment (infusion of acid) by altering their dietary choices, and only reduced their food intake when the disturbance to the rumen environment has been present for more than two hours. In contrast, when the sheep offered the B/P food choice, in which the low ED food was presented in a pelleted form, they markedly reduced their food intake and they did not alter their dietary choices. It is possible that the sheep on the first food choice (B/R) were under less metabolic stress to take the drastic step of reducing food intake in order to counteract the effect of the rumen infusion of acid than those offered than those offered the second choice (B/P), in which the alfalfa was pelleted.

The results from the tests carried on Chapter 4 and 5 to determine the effects of dietary carbohydrate source and physical form on diet selection were consistent with the proposal that the buffering capacity of the rumen could be maintained within certain limits by sheep modifying their feeding behaviour, and more specifically their dietary choice. It is likely that the manipulation of other physico-chemical attributes of foods will also affect the dietary choices of ruminants, this possibility will be considered in a later section.

*Direct manipulation of the rumen environment*

Through the use of intraruminal infusions in Chapter 3 (Experiment 2) and Chapter 5 it was possible to attempt to alter specific attributes of the rumen environment to test whether sheep will change their dietary choice in order to 'correct' the imbalance created, or at least to attempt to prevent any further deterioration. It was necessary to use sheep prepared with a rumen fistula in these experiments, but it is felt that the conclusions that can be drawn from experiments of this type can be used in the interpretation of the dietary choices made by
ruminants in general. Fistulation *per se* does not appear to affect the diet selection of sheep (Forbes & Beattie, 1987); nor was there any evidence that the housing or the experimental procedures used in Chapter 3 (Experiment 2) and Chapter 5 had any effect on diet selection or food intake; these results appear to be consistent with MacRae & Wilson (1977).

**Attributes of the rumen environment that appear to affect diet selection**

The materials, which were used to produce the rumen infusions administered in Chapter 3 (Experiment 2) (acid, alkali and saline) and Chapter 5 (acid), were expected to alter rumen pH and/or osmolality. It is known that the manipulation of these two attributes of the rumen environment has a significant effect upon the intake of single foods (Williams *et al.* 1987; Carter & Grovum, 1990a). Engku Azahan & Forbes (1992) have suggested that diet selection may also be affected by these two attributes of the rumen environment. In Chapter 3 (Experiment 2) and Chapter 5 the sheep, which were offered a choice of foods, responded to the direct challenges to the rumen environment by reducing the intake of the food which had the greater potential to adversely change rumen conditions, whilst maintaining intake of the other food offered. Engku Azahan & Forbes (1992) have also shown that when a rumen infusate is administered, sheep reduce the intake of a highly fermentable food, but maintain their intake of the low ED food offered.

The results from Chapter 3 (Experiment 2) appear to highlight rumen osmolality as a factor which influenced the dietary choice of sheep, as the intake of the high ED food tended to decline with increasing treatment osmolality, whereas the intake of the low ED remained constant. However the sheep studied in Chapter 5 appeared to alter their dietary choices in response to changes in rumen pH, rather than rumen osmolality. In Chapter 5 the sheep which were offered food choices containing a pelleted food (of high or low ED) paired with a relatively unprocessed food reduced their intake of the pelleted food when the rumen environment was challenged by an acid infusate. It is proposed that the intake of the pelleted
foods by the sheep was reduced in this instance, as such foods would not stimulate salivation, and thereby would not promote the buffering capacity of the rumen (Block & Shellenberger, 1980). Therefore it is possible that the nature of the food choices offered in Chapter 5 may have enabled the sheep to attempt to maintain the buffering capacity of the sheep. The food choice used in Chapter 3 (Experiment 2) wholly consisted of pelleted foods. This may have precluded the sheep from attempting to maintain the buffering capacity of the rumen by altering their diet selection as both foods had a low capacity to stimulate the salivation. As a consequence of this, dietary choices made by the sheep studied in Chapter 3 (Experiment 2) appeared to be consistent with an attempt to maintain rumen osmolality within certain limits, rather than rumen pH.

**Short and long term changes to the diet selection of sheep**

There is considerable evidence that ruminants can develop conditioned dietary aversions as well as monogastric animals (eg. Rozin & Kalat, 1971; Provenza et al. 1994). Thorhallsdottir et al. (1987) have shown that aversions which have been produced by pairing foods with LiCl can persist for at least 2 months. Animals can also exhibit conditioned food preferences, but these are frequently more difficult to establish and appear to be more transient than conditioned dietary aversions (Rozin & Zellner, 1985; Booth, 1985; Pliner et al. 1985). Results from Chapter 3 (Experiment 2), Chapter 5 and Engku Azahan & Forbes (1992) are consistent with the proposal that sheep can learn to associate foods with negative post-ingestive consequences that do not involve the emetic system. These linkages appear to be more transient than the conditioned dietary aversions that are commonly observed when LiCl is paired with foods, as the sheep reverted to their earlier dietary choices as rumen conditions returned to more typical levels.

Learned associations that are formed when the rumen environment is disrupted could be described as *partial aversions*, as the sheep which are offered a choice of foods appear to
respond minimising their consumption of the food which has the greater potential to adversely change the rumen environment. This term has been coined as it indicated that such associations elicit responses that are similar to full food aversions, but they are of a more transient nature. The more persistent effect of LiCl on dietary choice relative to HCl may stem from the fact that these two materials have different sites of action. The theoretical possibility that animals may exhibit some flexibility in the development of dietary habits has already been raised (Provenza & Balph, 1987). It is suggested that the results from Chapter 3 (Experiment 2), Chapter 5 and Engku Azahan & Forbes (1992) are the first examples of the development of partial aversions in ruminants.

THE RESPONSE TO A CHALLENGE TO THE RUMEN ENVIRONMENT: A PROPOSED MECHANISM

Observations made in Chapter 5 show that, when the rumen environment is challenged, the first response of sheep offered a food choice is to alter its diet selection by reducing the intake of the food which has the greater potential to change the rumen environment. If rumen conditions remain disrupted, the next response is to reduce total food intake. This second, more drastic response is the only strategy available to sheep which are given access to a single food, and it may be associated with some benefits, but obviously any reduction in food intake is be associated with costs. It is presumed that the objective of these responses is to 'correct' the induced imbalances, or at least to minimise further disruption to the rumen environment. Evidence which is consistent with this hypothesis can be drawn from Kyriazakis & Oldham (1993); in this experiment the sheep appeared to be attempting to avoid consuming high levels of non-protein nitrogen by altering their dietary choices, rather than by reducing their food intake. High levels of non-protein nitrogen are associated with high levels of ruminal ammonia.
(Fenderson & Bergen, 1976). The dietary choices of the sheep in Chapter 5 indicate that the ability of sheep to minimise further disruption to the rumen environment, by altering their dietary choices, can only occur if specific attributes of the rumen environment remain within certain limits. Once these limits have been exceeded, the only dietary strategy available to sheep to minimise further disruptions of the rumen environment is to reduce total food intake.

Monogastric animals such as pigs can make rapid and precise changes to their dietary choices (Kyriazakis & Emmans, 1990). It has been suggested that ruminants do not show the same degree of precision when making diet selections (Hou, 1991); in addition Provenza & Balph (1987) have suggested that the rumen can act as a buffer between the metabolism of the host animal and the foods available to it, thus enabling them to tolerate deviations from their target diet for a period of days, should circumstances require it. However, if changes in the available foods have a direct and possibly deleterious effect upon the rumen environment itself, there may be a need for the ruminant concerned to make a more immediate dietary response. The evidence presented in this thesis is consistent with the hypothesis that a mechanism exists which appears to operate to maintain conditions within the rumen within certain limits by altering feeding behaviour. This hypothetical mechanism is analogous with various homeostatic systems, such as that which maintains the concentration of glucose in plasma (Guyton, 1986); the possibility that the process of dietary choice may act in a homeostatic fashion has also been raised recently by Provenza (1995).

Provenza & Balph (1987) speculated that the process of diet selection in ruminants may be influenced by stimuli that are derived from receptors within the rumen. It has been demonstrated (Carter & Grovum, 1990b) that sheep will reduce their food intake within a matter of minutes when hypertonic rumen infusion is administered. Carter & Grovum (1990b) proposed that the only osmo-receptors that would be stimulated, within this time interval, would be sited in the rumen itself. As the sheep in Chapter 3 (Experiment 2), Chapter 5 and
those studied by Engku Azahan & Forbes (1992) rapidly altered their diet selections when the 
rumen environment was challenged, this may indicate that receptors that are sited in the rumen 
produce stimuli that have an influence on diet selection also. The rapidity of the response 
suggests that the hypothetical mechanism mentioned above is brought into operation swiftly, 
so that conditions within the rumen are restored to more typical levels. Furthermore, the 
obvious similarity of the dietary choices made by the sheep in Chapter 3 (Experiment 1) 
indicated that such a system can operate with a degree of precision.

ALTERATIVE HYPOTHESES TO ACCOUNT FOR THE FEEDING 
BEHAVIOUR OF SHEEP

In this study of the factors affecting diet selection in sheep most attention has been given to 
considering the significance of factors which are derived from the reticulo-rumen. In 
particular, rumen pH and rumen osmolality have been considered as factors which may affect 
the process of diet selection; evidence in support of this hypothesis has been drawn from 
Chapters 3, 4 and 5 and also from the work of Engku Azahan & Forbes (1992). It should be 
noted that it was not the intent of this thesis to ascribe the changes in feeding behaviour of 
sheep to changes in rumen pH and osmolality alone. To adopt such an approach would be 
naive, as it has been demonstrated (LeMagnen, 1985; Weston & Poppi, 1987; Forbes & 
Barrio, 1992) that changes in feeding behaviour cannot be related to changes in one factor 
alone.

Aspects of the rumen environment that may affect diet selection of sheep

Evidence presented in this thesis supports the proposition that changes in rumen pH and 
possibly rumen osmolality have an effect upon the dietary choice of sheep. It is recognised that 
other aspects of the rumen environment may also affect the process of dietary choice, some of 
these factors are outlined below.
Digestive processing constraints such as gastro-intestinal capacity and processing time are of considerable importance in determining both an animal's diet and its digestive strategy (Illius & Gordon, 1990; Penry, 1993). It has been proposed by many, such as Conrad et al. (1964), Van Soest (1982), Forbes (1985) and Poppi et al. (1994) that the volume of the gastro-intestinal tract imposes limitations upon the dry matter intake of large generalist herbivores. It is recognised that alternative hypotheses have been proposed (Grovum, 1987; Ketelaars & Tolkamp, 1991) which do not ascribe to the tenet that gut volume is a factor which limits food intake. Belovsky (1978) has proposed that the capacity of an animal to ingest 'bulk' may limit an animal's ability to forage in an optimal manner, therefore this constraint would limit the ability of the animal to maximise its rate of energy intake through its dietary choices. The capacity to ingest 'bulk' has been successfully employed by Belovsky (1978) as a constraint in a model of feeding behaviour which uses linear programming techniques. However, it should be noted that both Thompson Hobbs (1990) and Illius & Gordon (1990) have criticised the model of Belovsky (1978) because of its reliance upon 'bulk capacity' as a limiting factor. In these papers, it was judged that 'bulk capacity' was an inappropriate constraint; instead dry matter capacity has been proposed as an alternative which has more biological relevance (Thompson Hobbs, 1990; Illius & Gordon, 1990).

In ruminant species other digestive constraints are also of interest, specifically the rate at which particles leave the rumen. It is accepted that the rate at which material is able to pass out of the reticulo-rumen is a major factor which affects the intake of ruminants (Murdock & Wallenius, 1980; Van Soest, 1982; Forbes, 1985; Weston & Poppi, 1987). Malbert & Ruckebusch (1989) have proposed that the speed with which particles can pass out of the rumen- reticulum is limited by the rate at which material can pass through the pyloric sphincter, when this stricture is removed hyperphagia has been induced in sheep.
In their studies of feeding behaviour both Illius & Gordon (1990) and Milne (1991) have emphasised the rate of clearance of food from the reticulo-rumen as an important factor. It is proposed that passage rate is directly related to metabolic body size ($W^{0.27}$; Illius & Gordon, 1990). This relationship has been used by Illius & Gordon (1990) in a model of feeding behaviour, which relates the differences in the dietary choices of 'large' and 'small' generalist herbivores to differences in digestive morphology.

Within the same species, changes in physiological state can affect rumen outflow rate, for example pregnancy and lactation are associated with an increase in rumen outflow rate (Coffey et al. 1989; Weston, 1988). It is possible that the differences in rumen outflow rate that exists between young and old sheep (Weston et al. 1989; Leibholz, 1991) may be one of the factors which accounts for the effect of age on dietary choice that was observed in this thesis (Chapters 4 & 5). This possibility has been raised in an earlier section of the present chapter.

It is known that the manipulation of certain food attributes, such as physical form (Welch, 1982) and the addition of mineral buffers (Denholm & Ling, 1987) can alter rumen outflow rate. In this thesis these attributes of foods have been manipulated (Chapters 4 and 5 (effect of physical form); Chapter 4 (effect of sodium bicarbonate)) in order to determine whether this had any effect on dietary choice.

The effect of sodium bicarbonate on rumen outflow rate and diet selection

The inclusion of sodium bicarbonate is known to have a potent effect on the rumen environment, leading to increased rumen pH (Erdman, 1988) and increased rumen outflow rate (Denholm & Ling, 1987). An increase in food intake would be expected, when either rumen pH is increased (Mould & Orskov, 1984), or when rumen outflow rate is increased (Murdock & Wallenius, 1980). In addition to the above changes to the ruminal environment it
is also known that the inclusion of mineral salts in the foods offered to ruminants is associated with increased ruminal osmolality (Hart & Polan, 1984; Denholm & Ling, 1987); this effect on the ruminal environment is known to have an adverse effect upon food intake (Carter & Grovum, 1990a).

In Chapter 4 the effect of sodium bicarbonate inclusion on dietary choice was studied by incorporating bicarbonate into high-ED foods (1, 2 & 4 % (w/w)). In the light of the results reported by Ha et al. (1983), Mees et al. (1985) and Denholm & Ling (1987), it would be expected that sheep, offered such a choice, would select a diet that would maximise the advantages to be gained from the ingestion of bicarbonate (for example increased rumen pH and rumen outflow rate), whilst minimising the disadvantages (increased rumen osmolality). Furthermore, it is presumed that such a diet would facilitate high levels of food intake (Erdman, 1988). However, the results from Chapter 4 were not consistent with this hypothesis, as the sheep selected the same proportion of the supplemented food, irrespective of the level at which bicarbonate was added. Several explanations have been proposed in this chapter and Chapter 4 which may account for the fact that the sheep selected the same proportion of the supplemented food, regardless of the level of bicarbonate inclusion. A further explanation is now offered, namely that this behaviour was because the various effects of bicarbonate on the rumen environment had mutually antagonistic effects on the processes of diet selection, and as a consequence there was no net effect of level of bicarbonate on dietary choices reported in Chapter 4.

The effect of physical form of foods on rumen outflow rate and diet selection

It is well known that the intake of forages is increased when they are pelleted or chopped into short lengths (Owen et al. 1969; Welch, 1982). This effect of physical form on food intake has been related to the faster rate of passage of processed forages through the rumen (Welch, 1982; Murphy & Kennedy, 1993). The increased rate of passage of processed forage is due to
the fact that such treatment is known to increase the speed at which food particles can be reduced to a size that permits them to pass out of the reticulo-rumen (Beauchemin, 1991).

The forage preference studies of Kenney et al. (1984) and Hadjigeorgiou & Gordon (1994) are consistent with a hypothesis that diet selection is affected by differences in the rate of passage through the reticulo-rumen. In these experiments (Kenney et al. 1984; Hadjigeorgiou & Gordon, 1994) the sheep consistently selected the short chop forage, which would have a higher rate of passage (Murphy & Kennedy, 1993), in preference to long chop forage. It is possible that differences in the ease of communication of stem and leaf tissue (Murphy & Kennedy, 1993) may be one of the factors that accounts for the preference of sheep for young leaf tissue over more lignified stem tissue (Arnold, 1966; Milne, 1991). However, evidence from Chapters 4 and 5 indicates that differences in rumen outflow rate that are due to the physical processing of foods cannot solely account for the effect of physical form on dietary choice. In both of these chapters sheep were offered a choice between pelleted alfalfa and the same material left unprocessed, these animals did not select the pelleted alfalfa alone, instead they consistently selected a mixture of both foods. Had these animals selected a diet consisting of the pelleted alfalfa alone it is presumed such a diet would be associated with a high rumen outflow rate (Otjen et al. 1965), and that this permitted the high rates of intake that were observed when this food was offered alone (Chapters 4 and 5).

Differences in the passage rate of grass and clover (Greenhalgh, 1979) do not appear to have been the sole factor directing the dietary selection of grazing sheep offered a choice between grass and clover. Parsons et al. (1994) have observed that sheep do not select a diet consisting of clover alone, despite its higher passage rate and higher rate of potential intake. In general, differences in rumen outflow rate can have a considerable effect on food intake, and it is probable that this factor does have a considerable effect on dietary choice. It is unlikely,
however, that differences in the rumen outflow rate can account for the effect of physical form on diet selection, to the exclusion all of other factors.

*The significance of a fibrous mat within the reticulo-rumen*

It has been proposed (Chapters 4, 5 and the present chapter) that the selection of unprocessed forage indicates that such foods have beneficial properties, which are either not possessed by more processed forages, or not to the same degree. It is recognised that the provision of low-ED unprocessed foods stimulates chewing behaviour, and thus salivation. Two attempts have been made to categorise forages according to this attribute (i.e. Balch, 1971; Sudweeks et al. 1981). It has already been proposed (Chapters 4, 5 and present chapter) that one factor which affects diet selection is the ability of unprocessed forages to enhance the buffering capacity of the rumen, by eliciting the production of saliva (Turner & Hodgett, 1955). The ability of unprocessed forages to form a fibrous mat within the rumen is also a factor which may have an effect on dietary choice. It is known that the presence of a fibrous mat would promote ruminal development (Block & Shellenberger, 1980; Murdock & Wallenius, 1980) and would minimise the incidence of disorders such as acidosis and laminitis (Marshall et al. 1992; Feng et al. 1993). The significance of this property of many low-ED foods has been alluded in the Introductory section of Chapter 3. It should be noted that there has been no systematic attempt to consider whether the ability of low ED foods to form a fibrous mat is of sufficient significance that an animal would alter its dietary choices to obtain such material.

It has been shown that the pH of rumen contents is a major factor influencing the level of food intake (Mould & Orskov, 1984; Williams et al. 1985; Rymer & Armstrong, 1989). This evidence suggests that the buffering capacity of unprocessed forage does have an effect upon dietary choice and the results from Chapter 3, experiment 2, Chapter 5 and from Engku Azahan & Forbes (1992) appear to support this proposition. However, it remains to be seen
whether it is the high buffering capacity of low-ED foods or their ability to form fibrous mats in the reticulo-rumen that influences the diet selection of sheep.

\textit{Diurnal effect of diet selection}

The studies of the grazing behaviour of sheep (Parsons \textit{et al.} 1994) and cattle (Penning \textit{et al.} 1995) on grass/clover swards have shown that the dietary choices of ruminants alter during the course of a 24-h period. Both Parsons \textit{et al.} (1994) and Penning \textit{et al.} (1995) reported that the observed animals showed a preference for clover in the morning, switching to the selection of grass in the late afternoon and early evening.

Two explanations are outlined which may account for this behaviour. Parsons \textit{et al.} (1994) suggested that the strong diurnal pattern of dietary preference indicates that an ingestive strategy may exist. It is known that sheep spend longer ruminating after eating grass than after eating clover (Penning \textit{et al.} 1991); this behaviour occurs mainly at night, when grazing is minimal or absent (Lynch \textit{et al.} 1992). Parsons \textit{et al.} (1994) proposed that the selection of grass toward the end of the day permitted this material to be fully digested without impeding further consumption. Alternatively, the diurnal patterns of dietary preference that were observed by Parsons \textit{et al.} (1994) and Penning \textit{et al.} (1995) may be due to temporal changes in the characteristics of the grass and clover. Plant dry matter content shows diurnal variation, being at a minimum during the evening and reaching a maximum at midday (Street & Opik, 1976; Heath & Hebblethwaite, 1987). These diurnal changes may magnify inter-species differences in the concentration of nutrients. It is speculated that the shift in dietary preference reported by Parsons \textit{et al.} (1994) and Penning \textit{et al.} (1995) may be a response to these hypothetic diurnal changes in plant composition.
An attempt was made to record the feeding behaviour of sheep over a 24-h period in Chapter 5. There was no evidence to indicate that the sheep demonstrated any diurnal variation in their dietary choice, as the diet selected by the sheep during the 12 hours of light (0700-1900 h) did not significantly from the dietary choices made during the 12 hours of darkness (1900-0700 h). The proportion of pelleted food (food B or P) that was selected by the sheep between 0700-1900 h was not significantly different from the diet selected between 1900-0700 h), being 622 & 670 g/kg total food intake (SED 95.8; P>0.05) respectively. Kyiazakis & Oldham (1993) have also observed that the diet selections of sheep kept in similar circumstances do not show diurnal variation.

It should be noted that the sheep in Chapter 5 maintained a diurnal pattern of feeding behaviour, whilst housed in metabolism crates which was similar to the feeding behaviour of sheep in less controlled environments (Lynch et al. 1992; Chiofalo et al. 1992). The maintenance of a typical ovine feeding pattern by the sheep in Chapter 5 suggests that the mode of housing that was used does not account for the absence of a diurnal pattern to the dietary choice. No reason is proposed to account for the absence of a diurnal pattern to the dietary choices of the sheep in Chapter 5, as opposed to the observations of Parsons et al. (1994) and Penning et al. (1995). Nevertheless, it is possible that a diurnal pattern may have been observed, if it had been possible to make more frequent, focal measurements of food intake, using an automatic intake-recording system such as those described by Anil et al. (1986) and Neilsen et al. (1993).
The significance of sampling behaviour in diet selection

It is often observed (Malechek & Balph, 1987; Provenza & Balph, 1990) that sheep and other animals eat, at regular intervals, small quantities of all available foods. It is believed that the objective of such behaviour is to maintain current knowledge of all available foods (Illius & Gordon, 1990), and thereby facilitate future choices. Sampling behaviour is not without its inherent costs, this has been acknowledged by Illius & Gordon (1990) and Westoby (1978), who have proposed that the maintenance of current knowledge of all available foods by sampling may explain why large, generalist herbivores, such as sheep frequently select a diet that appears not to be optimal.

A clear example of sampling behaviour is witnessed when sheep (Chapple & Lynch, 1986; Burritt & Provenza, 1991) and other animals (Rozin, 1976) encounter novel foods, in such circumstances they are known to cautiously sample small quantities of the new foods. If no adverse consequences ensue the animals concerned will increase consumption, but if they become ill or there are other adverse consequences, the animals will reject the new food (Burritt & Provenza, 1991). In the experiments that comprise this thesis and those of others, such as Kyriazakis (1989) and Bradford & Gous (1991) the animals were given the opportunity to overcome their food neophobia during a training period. It is believed that by following such a regime, animals have the opportunity to overcome their neophobia by learning about the foods and the consequences of consumption (Kyriazakis, 1989).

Even in the relatively controlled environments that were reported in this thesis and those of Kyriazakis & Oldham (1993) and Chapple et al. (1987) it is probable that the sheep were exhibiting sampling behaviour; this point has been raised in Chapters 3 & 4 and also by Kyriazakis & Oldham (1987). Provenza & Balph (1990) have proposed that sheep continue to sample familiar foods in familiar environments as changes can occur to foods over time, that
may warrant a change in dietary choice. Such temporal changes may include changes to nutrient contents (Shallenberger & Moyer, 1961; Street & Opik, 1976) and changes to the presence and concentration of anti-nutrients (Hegarty, 1982; Provenza et al. 1994).

Although sampling is an important aspect of diet selection, it is unlikely that the selection of mixed diets by sheep is due to sampling behaviour alone. A few points are raised below which appear to support this proposition. Firstly, Parsons et al. (1994) have proposed that unless information has to be updated continuously, sampling cannot explain the fact that sheep consistently select mixed diets. This proposal is reinforced by some results that have been reported by Edwards et al. (1994), who have demonstrated that sheep are able to remember the spatial location of foods and that they will use that information in further exposures to the same environment. Secondly, if sampling behaviour were the sole explanation of the selection of mixed diets by sheep, this implies that sheep follow a feeding pattern that has been described by Rozin & Kalat (1971) as 'continuous avoidance'. Such a strategy assumes that animals eat from one food until adverse consequences ensue, whereupon they switch to the other food or foods offered (Rozin & Kalat, 1971). It has already been mentioned (Chapter 3, experiment 2) that the feeding behaviour of sheep does not appear to follow such a pattern (Zahorik & Houpt, 1977), this is re-inforced by later experiments in this thesis (Chapters 4 & 5) and also by Engku Azahan & Forbes (1992). The existence of a diurnal pattern to the diet selections of the sheep which were observed by Parsons et al. (1994) and Penning et al. (1995) also casts doubt over the proposition that sampling behaviour alone can account for the selection of mixed diets by sheep. Finally, when sheep choose to include 30% of the less preferred food in their selected diet, it seems probable that factors other than the need to maintain current knowledge may direct diet selection.
The effect of the senses on diet selection

It has been demonstrated that sheep have excellent visual acuity, which enables them to distinguish familiar faces from nonfamiliar ones (Kendrick & Baldwin, 1987). Cahn & Harper (1976) have suggested that sheep are able to distinguish between different clover polymorphisms on the basis on the white leaf marks on these plants. Some analogous work has been conducted by Mottershead et al. (1985), who have shown that sheep can discriminate between whole-grain wheat and barley. Sheep have an inability to see in colour (Tribe & Gordon, 1949), but they are able to use other visual cues such as height and luminance (total amount of incoming radiation reflected from an object (Uvarov et al. 1984)) to distinguish between different sward types (Bazely, 1991).

The olfactory senses of sheep are equally developed, as Bell (1959) demonstrated that ruminants can discriminate between salty, bitter, sour and sweet tastes. Sheep are also able to distinguish between foods that have been permeated with faecal odours from those which have been similarly treated with oil of wintergreen (Pfister et al. 1990). Although the sense of touch has not been studied in a systematic factor (Lynch et al. 1992), it is probable that tactile stimulation of the muzzle is also of importance, as sheep tend to avoid plants with spines and stinging hairs (Provenza & Balph, 1990).

The work of Bazely (1990) indicates that sheep do not make dietary choices according to visual differences alone. Bazely (1990) demonstrated that the preference of sheep for dark green rather than light green herbage was not due to a preference for optically dense herbage per se. Instead, these sheep were using variations in brightness as a highly reliable cue to the concentrations of nitrogen and water-soluble carbohydrates.
From an evolutionary perspective there is some benefit to be gained from selecting sweet
tasting foods, as these are frequently a source of energy, and in avoiding bitter tasting foods
(Rozin & Vollmecke, 1986). Provenza & Balph (1990) have suggested that the studies of
conditioned taste aversions and preferences suggest that the palatability of foods is not
absolute, instead an animal's response to a food flavour is dependant upon post-ingestive
consequences. However, nutritional value does not always relate to the taste of the food
(glucose and saccharin), and it has been demonstrated (Burritt & Provenza, 1992) that sheep
can learn to show a preference for flavours paired with glucose, rather that those which are
paired with saccharin. Secondly, Blair & Fitzsimons (1970) have demonstrated that the
response of animals to bitter tastes is not fixed, as they have shown that animals offered foods
flavoured with extremely bitter flavours (Bitrex), overcome an initial aversion to the treated
food and subsequently maintain a level of intake that is comparable with the control animals.
Furthermore, Nolte & Provenza, (1992) have shown that the flavour preferences of lambs
only persist when the animal has learnt to associate the flavour with positive-ingestive
consequences.

The work of Bazely (1990), Nolte & Provenza (1992) and Blair & Fitzsimons (1970),
amongst others, indicates the olfactory and visual senses produce stimuli that act as a cues.
Provenza & Balph (1990) have proposed that animals use sensory differences of taste, smell
and texture as cues to enable them to distinguish between different foods, and this permits
them to make appropriate dietary selections (Provenza & Balph, 1990).

In all of the experiments described in this thesis and the experiments reported by several others
animals have been given a training period in which foods are offered singly, in an alternating
pattern, to allow the animals to learn to associate the sensory differences between foods with
post-ingestive consequences. The following sensory cues have been used deliberately: position
(Chapters 1, 2, 3, 4 & 5; Kyriazakis & Oldham, 1993), colour (Kutlu & Forbes, 1993) and taste (Dalby et al. 1994a). It is also possible that animals may use texture, and smell as further sensory cues (Pfister et al. 1992). The provision of a training period confers benefits as animals which have followed such a regime select an apparently balanced diet more rapidly than those which have not (Dalby et al. 1994b). Furthermore, Edwards et al. (1994) have demonstrated that prior experience enables sheep to locate food patches more rapidly.

In summary, the senses of taste, smell, touch and sight are of importance in the diet selection process (Chapple et al. 1987). There is considerable evidence which indicates that sheep do not select diets with the sole objective of gaining pleasurable sensory stimuli, (Provenza & Balph, 1990), instead such stimuli is used as a cue which enables sheep and other animals to distinguish between foods, and make their dietary choices.

**ISSUES RAISED AND FUTURE WORK**

*Interaction between physiological state and dietary constraints*

Penry (1993) has proposed that the dietary strategies of an animal must operate within the framework of its gastro-intestinal physiology. It is likely that differences in the rumen capacity and the ability to utilise fibrous foods can account for the effect of age on diet selection which was described earlier in this chapter. The diet selections by the ewes in Chapter 2 which were offered a low ED food gave rise to the possibility that dietary constraints or other objectives may supercede the priorites of the animal's physiological state in some circumstances. One can envisage other examples in which the effect of physiological state would be modified by dietary constraints, specifically those which are associated with the reticulo-rumen. For example when ruminants are close to parturition they have a high requirement for energy and
protein (ARC, 1980: AFRC, 1992). However in this pariparturient state the buffering
capacity of the rumen is minimal (Counette et al. 1979), this metabolic stress creates a
dilemma for the animal as the low buffering capacity of the rumen may curtail the ability of
the animal to select a diet that is appropriate to its requirements.

Environmental constraints may also affect the ability of ruminants to select a diet that is
appropriate to their physiological state. It is already known that ambient temperature can be
such a constraint (Cropper & Poppi, 1992). Although sheep have a considerable ability to
'store' water and to conserve it (Shkolnik et al. 1980), there are circumstances where the
drives of hunger and thirst may come into conflict (Lynch et al. 1992), and it is likely that the
availability of water may act as a constraint upon diet selection of sheep. For example, the
restricted availability of water may prompt sheep, which are offered a choice between foods of
different DM concentration, to select a diet that is not necessarily appropriate to their
physiological state.

It is possible that social constraints may also thwart the ability of ruminants to select a diet
that is appropriate to their physiological state, as it is already known that animals which are
low in the dominance ranking of a group have less opportunity to gain access to food (Lynch
et al. 1992) than those which have a higher ranking. This hypothesis could be tested by
offering to sheep, housed in single-sex groups, a choice between foods of high and low ED or
foods of high and low crude protein content, where the access to one or both of the two foods
is limited to one sheep at a time. The use of a computerized single-space feeding system, such
as that described by Neilsen et al. (1993), would ensure that a study of the effects of social
constraints on diet selection of ruminants could be made, without sacrificing the opportunity
to collect intake data from individual animals.
Physico-chemical attributes of foods

There appear to be many factors which impinge upon the mechanism which regulates dietary choice. The mechanism which regulates food intake is considered to be a multifactorial system (Forbes & Barrio, 1992; Mbanya et al. 1993). Although it is possible that the mechanism which regulates diet selection is also of an additive nature, it is more likely that the process of diet selection is directed by a 'desire' to meet a series of ranked objectives, such as rapid growth and successful reproduction (Emmans & Kyriazakis, 1994). Furthermore, it is probable that as the available foods alter, the order of these objectives changes (Emmans, 1991). For example, the energy density of the food choice offered to pregnant ewes (Chapter 2) appears to determine whether their dietary choice was directed by a 'desire' to satisfy the energy and protein requirements of this state or a 'desire' to maintain a balanced rumen environment.

It is accepted that the factors which act upon the process of diet selection are derived from several sources (Provenza & Balph, 1990). It is recognised that pre-ingestive factors such as sward structure, plant height and plant anatomy have an effect upon dietary choice of ruminant (Hodgson, 1982; Milne, 1991). The taste, odour and texture of the ingested food provide stimuli which also act upon the process of dietary choice (Gherardi et al. 1991), although taste may be important, sheep can learn to ignore such stimuli if the different food flavours are not associated with different post-ingestive consequences. The evidence from a number of diet selection experiments (Cropper, 1987; Kyriazakis & Oldham, 1993; Chapter 3 (Experiment 1); Chapter 4) is consistent with the proposal that nutritional qualities of foods, such as the concentration of protein, are sensed by visceral receptors and that the stimuli have an effect upon dietary choice of ruminants. It is suggested that the significance of these various stimuli depends on the time-scale which is being considered, organoleptic stimuli may
direct minute by minute selections, but visceral stimuli probably direct long-term diet selection.

Results presented in this thesis and a number of other experiments (Engku Azahan & Forbes, 1992; Parsons et al. 1994) are consistent with the proposal that diet selection is influenced by physico-chemical attributes of foods which can affect conditions within the rumen. It is known that varying dietary carbohydrate source (Van Soest et al. 1991) and the physical form (Marshall et al. 1992) of foods can affect the rumen environment; the results from Chapters 4 and 5 indicate these two food attributes have an effect upon diet selection also. The study of the effect of physical form on diet selection in Chapters 4 and 5 used alfalfa in two markedly different forms. The results suggest that the ability of foods to elicit chewing behaviour is a positive attribute. To extend this study it would be worth considering other foods which can be presented in different physical forms. For example one could envisage offering to ruminants, a choice between high- and low-ED foods, where the low-ED food had either a high or low cation exchange capacity (CEC), as McBurnley et al. (1983) has shown that there is a high correlation between CEC and buffering capacity. It is possible that this attribute of low-ED foods may of significance in determining dietary choice.

Phytotoxins are produced by many of the plant species that are consumed by ruminants (Hegarty, 1982; Malechek & Provenza, 1987). These toxins can be divided into those which cause gastro-intestinal distress (bloat: high concentrations of soluble leaf protein (McDonald et al. 1988) (many forage legumes)) or impaired digestion (reduced microbial protein production: condensed tannins (McNabb et al. 1993) (Sainfoin, Onobrychis vicifolia)), and those toxins which have a more systemic effect (goitre: glucosinolates (Hegarty, 1982) (Kale, Brassica oleracea)). Duncan & Milne (1993) have shown that the voluntary food intake of sheep is depressed when they receive, by oral administration, brassica secondary metabolites that are known to have a detrimental effect on the liver and kidneys. To survive and grow in
an environment which contains plants that produce phytotoxins, it is necessary for the metabolism of ruminants to adapt to cope with or tolerate the ingested toxins, or for the animal concerned to learn to associate foods with the post-ingestive consequences of eating them (Provenza et al. 1993b). Duncan & Milne (1989) have demonstrated that lambs can tolerate the presence of brassica-derived gluconsinolates in the foods offered to them, after a period of exposure. There is also some evidence (Provenza et al. 1994) that ruminants (goats) will acquire a preference for old growth from the shrub Blackbush (Coleogyne ramossima) rather than new growth from the same species because the young plant tissue contains higher levels of condensed tannins, relative to the older tissue. This is despite the fact that the younger tissue has a high concentration of protein.

Sheep can exhibit long-delay learning as they are able associate the administration of LiCl with a novel food that was consumed up to 6 hours before (Bun-itt & Provenza, 1991). However there appears to be no evidence to indicate that sheep demonstrate long delay learning when the other media are used, such as phytotoxins. This possibility merits investigation. There is some evidence that pigs will attempt to minimise their intake of gluconsinolates, which have a deleterious effect on the general metabolism, when offered a choices between a food with a high glucosinolate content and one with a lower content; this desire to minimise the level of this toxin in the selected diet appears to supercede other objectives, namely rapid growth (Kyriazakis & Emmans, 1992). It would be worth considering whether ruminants are also able to alter the dietary choices to cope with the dietary inclusion of phytotoxins that exert a more systemic deleterious effect on metabolism.

*The dietary response to induced disturbances to the rumen environment*

Evidence presented in this thesis suggests that infusions into the rumen that cause rumen pH to be changed are of sufficient importance to sheep that they will alter their dietary choices in
the short term in an attempt to 'correct' the imbalances, or at least to minimise further disturbances. However, it is acknowledged that the manipulation of rumen pH does have some effect upon other attributes of the rumen environment and it is difficult to disentangle these various interactions.

Kyriazakis & Oldham (1993) have suggested that sheep will attempt to select a diet that enables them to avoid consuming high levels of non-protein nitrogen, which would be degraded to form ammonia in the rumen. The dietary choices of the ewes in Chapter 2 are consistent with the hypothesis that sheep will attempt to avoid an asynchronous supply of energy and protein to the rumen. However, in this thesis there has not been the opportunity to give further consideration to this hypothesis. High levels of ruminal lactic acid are also detrimental to ruminants (Dirksen, 1970) and can depress food intake (Smith et al. 1979; Critchlow, 1988). It is possible that in Chapter 4 the sheep which were offered food choices containing barley predominantly selected more of the low-ED food offered than those offered the SBF/barley choice, in an attempt to 'dilute' the lactic acid that would be produced from the ruminal fermentation of the barley-based food. At present, this remained a speculative issue as no measurements of ruminal lactic acid levels were made. However, it may be worthwhile considering the possibility that sheep will alter their diet selections to ensure that ruminal lactic acid levels are maintained below certain levels.

In this work, mineral acids and alkalis have been used to alter conditions within the rumen to determine whether sheep will alter their diet selection in the short term to 'correct' imbalances. There is considerable interest in the effect of polyether ionophores on metabolism, growth and body composition of ruminants (Owens et al. 1991). Ionophores such as monensin have a potent effect upon rumen fermentation (Jouany, 1994). These compounds exert a negative selection pressure on Gram negative bacteria, which have cellulytic activity (Ketchum, 1988), as a consequence the dietary inclusion of monensin and other such compounds are associated
with an increased production of propionate at the expense of acetate. In addition, the use of monensin has other benefits, such as lessening the incidence of bloat and acidosis (Owens et al. 1991). Given that the use of ionophores can exert these physiological effects it seems likely that the use of these compounds may cause ruminants, which are offered a choice of high- and low-ED foods, to reduce their selection of the low ED food offered as they would have a reduced capacity to utilise this food.

The dietary response that is made by sheep when the rumen environment is disrupted appears to be both rapid and precise. The first response that is made by sheep offered a choice of foods is to alter their diet selection, to 'correct' the induced imbalance, and then if rumen conditions remain disturbed, to reduce food intake. It is possible that there is a point of inflexion along the continuum of rumen pH, above this point sheep will change their dietary choices to restore conditions within the rumen, and if rumen pH falls below this point, sheep will reduce their food intake. Further studies in which the rumen environment is directly manipulated are needed to determine whether such a point of inflexion exists, and to determine whether the nature of the foods offered to sheep affect the position of this site.

CONCLUSIONS

1 Sheep can exhibit state-dependant diet selection, but dietary constraints may ameliorate their ability to choose a diet that reflect their physiological state.

2 Dietary choice is affected by food attributes that would have an effect upon the rumen environment (the carbohydrate source in a food and its physical form). The lack of effect of dietary bicarbonate on diet selection can be ascribed to the type of training period used.
Changes to conditions within the rumen have a swift effect on feeding behaviour. Sheep appear to alter their feeding behaviour in an attempt to 'correct' any imbalances. The first response is to alter their diet selection, and if rumen conditions remain disrupted to reduce their food intake. Such changes in feeding behaviour are of a limited duration, and do not have a persisting effect upon dietary choice after rumen conditions have return to more typical levels.
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