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Nutrient selection by fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*)

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ABSTRACT

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NUTRIENT SELECTION BY FALLOW DEER (DAMA DAMA) AND ROE DEER (CAPREOLUS CAPREOLUS)

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Nutrient selection by fallow and roe deer was investigated both in the natural environment (southern Hampshire, UK) and experimentally (deer parks). Diet composition (faecal analysis), availability and quality (nitrogen, calcium, magnesium, potassium and phosphorus content) were measured for both species of deer, which are classified as different types of feeders. Diet selection in the natural habitat was investigated by combining these measures. Feeding trials were performed to test the discriminatory abilities of the deer based upon diets of different protein content.

The plant species present in the diet of both species of deer were found to depend on habitat, although the quantities eaten did not depend on availability. Seasonal variation in the intake of plant species was explained mainly by the life cycles of the ingested plants. Seasonal variation was also evident in the nutrient concentrations of ingested plants. Nitrogen, magnesium potassium and phosphorus peaked in concentration in late spring/summer, whereas the opposite pattern was found for calcium. Deciduous plants were found to have the greatest seasonal variation in comparison to evergreen species, due to differences in life cycles. Nutrient concentrations also differed between sites which might be explained by site differences in soil chemistry.

Diet selection in the natural environment was apparent from comparisons of amounts eaten to availability of the plant species. Selection was more evident in summer than winter and by roe more than fallow deer. The plants eaten in the highest proportions in the winter were also the most available at that time. These plants contained sufficient nutrients to meet the requirements of the deer, suggesting that in winter diets were not nutrient deficient. In summer more diverse plant species were eaten but the diets taken were deficient in crude protein and calcium, particularly for the female deer. This is the period of parturition and lactation, when nutrient requirements are at their highest. In some cases the plant species eaten could provide adequate nutrients but the deer did not take them in sufficient amounts to fulfil their requirements. Male fallow deer were also deficient in calcium during the period of antler regrowth in the summer, when the concentration of this nutrient was at its lowest. Male roe deer did not face the same difficulty as their antlers grow in winter when there was adequate calcium available. Overall, roe deer preferred vegetation of higher protein content, particularly in winter, suggesting selection may have taken place based on protein levels.

Feeding trials showed that fallow and roe deer have the ability to discriminate between two foods of different crude protein content. Learning was strongly indicated as both species of deer switched from one food to the other, usually after the first day of the trial, suggesting that post-ingestive feedback mechanisms were operating. The trials implied that protein content may have been the basis for the selection of the specific test diets. Roe deer selected higher protein diets than fallow deer. This discriminatory ability may extend to other nutrients, offering credence to the apparent diet selection findings in the natural environment.

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CHAPTER 1 INTRODUCTION

One of the most basic functions an animal has to perform is to feed. To do so most animals must forage. Foraging behaviour differs depending on the animal and its habitat. For many their environment is also continually changing. It is generally accepted that ruminants do not forage at random, but select a diet from the available vegetation (Provenza and Balph, 1990). Researchers have been attempting to understand diet selection and foraging behaviour for many years, and it is now recognized that there are essentially two explanations, although these are by no means mutually exclusive.

Firstly, functional explanations for foraging behaviour (*e.g.* optimality theory) look at what animals should do in certain circumstances, they define selection rules in terms of final outcomes (Staddon, 1983). Therefore they are concerned with behaviours that signify goals but do not consider the adaptive processes involved in reaching these goals. Secondly, causal or mechanistic explanations for behaviour (*e.g.* learning) answer how animals select and forage. These latter explanations define the mechanisms by which behaviours are generated and through which adaptive variants are selected (Staddon, 1983).

Functional and mechanistic explanations for diet selection have been investigated by researchers in different ways. Animals may be simply observed foraging and descriptions made of what was taking place. Following on from such studies, hypotheses can be created and models devised (Section 1.3.3) to further explain what animals would do in a given set of circumstances. Finally these explanations can be examined experimentally, for example by testing foraging theories (Belovsky, 1978, 1981: Section 1.3.3.5) or by more controlled feeding experiments (Section 1.3.3.4). Thus there are observational and experimental approaches to unravelling diet selection in animals.

This study was designed to investigate diet selection in fallow (*Dama dama*) and roe deer (*Capreolus capreolus*) in the south of England using both observational and experimental approaches. Until very recently diet selection has been poorly studied in herbivores, particularly ruminants. Researchers are becoming increasingly interested in the functional and mechanistic explanations for ruminant foraging behaviour. Much of this work has been done on ruminants such as goats (*Capra hircus*) and sheep (*Ovis aries*), very little has been done with deer especially with fallow and roe deer. These species of deer were chosen as they are classified as different kinds of feeders: concentrate selectors and intermediate feeders (see Section 1.2.4 for descriptions), and would therefore provide comparative data in terms of diet selection.

The observational approach involved the use of microhistological analysis of faecal material (Chapter 2), surveys of the availability of vegetation (Chapter 3) and chemical analysis of the forage material (Chapter 4). The experimental approach employed feeding trials in the form of preference tests (Chapter 6).

The aim of the observational approach was to investigate diet selection in the natural environment by looking at the vegetation the deer had been eating in relation to its chemical composition (macronutrients[1]: nitrogen, calcium, magnesium, potassium and phosphorus) and availability in the local environment over a year (Chapter 5). To then conclude whether or not selection for specific plants had been made on the basis of particular chemical components (*e.g.* protein, calculated from the nitrogen content) taking into consideration availability. This part of the study, therefore, looked at functional explanations for diet selection as it considered the final outcomes.

The availability of vegetation (Chapter 3) is of great importance, it must be considered at every step as diet selection could easily be due to plant availability irrespective of its nutrient value. Despite its importance, measuring the availability of vegetation is agreed by many to be a difficult task and is dependent on the habitat being surveyed and the study animal (Bobek and Dzieciolowski, 1972; Perzanowski, 1990; Weckerly and Kennedy, 1992).

There have been few published accounts of the chemical composition of plants eaten by deer. Many of these have only investigated the nutrient content in one season, usually winter as this is when most nutrients are limiting to the deer (mule deer *Odocoileus hemionus*: Smith, 1957; black-tailed deer *O. hemionus columbianus* Radwan and Couch, 1974; white-tailed deer *O. virginianus*: Hellmers, 1940; Torgerson and Pfander 1971) or at one specific time of year (white-tailed deer: Swift, 1948). One of the most comprehensive descriptions of the nutrient contents of plants was carried out by Dietz *et al* (1958, 1962), they looked at the principal browse species in the diet of mule deer. However, this detailed analysis was conducted in an area of Colorado, USA, where the terrain and climate are very different to that of this study in southern England. Similar work carried out in the locality of this current research is limited to Ekins (1989). She investigated the forage resources of cattle (*Bos taurus*) and ponies *(Equus caballus)* in the New Forest including the qualitative (chemical) analysis

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[1] macronutrients are nutrients (chemical elements) required in large quantities by plants and animals, as opposed to micronutrients which are required in smaller amounts.

of vegetative samples. Although her work did not look specifically at the diet of the deer in the New Forest, it did include nutrient analysis of plant species commonly found in the diet of the deer in this study. Some work has looked specifically at the chemical content of plant species eaten by fallow deer in the New Forest, but unfortunately it has not been published and is only referred to in Putman (1986a).

Combining the chemical composition of many forage species with their importance in the diet to investigate diet selection has been done by a few researchers (*e.g.* Ramírez *et al*, 1996), but this current study is the first to look at this in two comparative feeders and in such great detail over an entire year. Even less work has been done experimentally to look at diet selection in deer (*e.g.* Gillingham and Bunnel, 1989) and none have used fallow and roe deer. The current study used controlled feeding trials more usually associated with rats than ruminants. Although diet selection in the natural environment will consider several macronutrients, the feeding trials could only be designed to investigate one in detail, protein was chosen, the reasons for which are given below.

The feeding trials (experimental approach) addressed the mechanistic explanations of diet selection. The trials were designed to see if the deer could detect differences in diets that differed in one major component (protein) and to then hypothesise as to the mechanisms that could be operating (*e.g.* learning, post-ingestive feedback). The trials (Chapter 6) were carried out using a preference test procedure similar to those used extensively for testing rats (*Rattus norvegicus*: *e.g.* Baker and Booth, 1989a) and also cats (*Felis catus*: *e.g.* Hegsted *et al*, 1956). An important factor in preference tests is that the foods taste or smell differently to allow the animals being tested to distinguish them. In most cases diets are made up so that one of them is more attractive (*e.g.* more palatable) and this is not the one the animals are expected to eventually choose. In general terms the aim of the preference test is to 'force' the animal to take the more attractive food first, thus enabling the researcher to see a switch to the other food, or as in most cases to a mixture. In situations where the diets are very similar and there are no distinguishing sensory stimuli, artificial flavours are used, thus allowing the animals to associate particular qualities of the food with a particular flavour.

Protein was chosen as the main nutrient to investigate diet selection in fallow and roe deer as it has been suggested by Parfitt (in Putman, 1986a) to be a limiting factor in the diet of deer, particularly over the winter. The nitrogen content of plants can also be easily and reliably measured, and then converted into protein using an accepted constant (see McDonald *et al*, 1981). Several studies have investigated the protein requirements of deer, in particular the white-tailed deer in North America, and moreover the specific requirements of fawns and yearlings (French *et al*, 1956; McEwan *et al*, 1957; Dietz, 1965; Murphy and Coates, 1966; Ullrey *et al*, 1967; Smith *et al*, 1975; Holter *et al*, 1977, 1979). However, there is very limited information on the protein requirements of fallow deer, the only known study was by Putman (1980) looking at protein intake in fallow deer fawns. The only other source for fallow deer is Reinken *et al* (1990); guidelines for deer farmers. There are no known published requirements for roe deer, probably because they are not farmed. The data obtained in the current study will provide evidence for whether or not the published requirements for fallow deer are firstly available and secondly being taken. It will suggest requirement levels for roe deer.

Although some researchers have described deer diets and others have chemically analysed plant material that is eaten, none have brought these two techniques together to look at diet selection in deer and in particular fallow and roe deer.

Firstly the natural history of fallow and roe deer will be described (Section 1.1). This is followed by an account of the digestive physiology and metabolism of ruminants in general (Section 1.2) which includes the classification of fallow and roe deer into their different feeding types (Section 1.2.2). The concept of diet selection is then introduced and models used to understand how and why animals feed selectively are investigated (Section 1.3). Finally the aims and rationale behind the thesis are provided in Section 1.4 and consider the main questions asked in each chapter.

1.1 DEER

Deer belong to the family *Cervidae* and are artiodactyl ungulate ruminants (Class: *Ungulata;* Order: *Artiodactyla;* Suborder: *Ruminantia*). Forty different species make up the *Cervidae*, which occur throughout Europe, Asia, the Americas and parts of Africa. They occupy a variety of different habitats from marsh to tundra. There are five subfamilies, the main ones (containing the most species) being the *Cervinae* and the *Odocoilinae.* Table 1.1 lists examples of deer species within the five subfamilies.

1.1.1 Fallow deer

History and distribution

Fallow deer belong to the genus *Dama* in the subfamily *Cervinae* (Table 1.1). Two sub-species exist,

Table 1.1 The five subfamilies within the family Cervidae, examples of species and their distribution are given including fallow deer and roe deer in bold (adapted from Putman, 1988).

Subfamily	**Species include:**	**Distribution**
Moschinae: Musk deer	Siberian Musk deer *Moschus moschiferus*	Russia, Korea, northern China *(3 subspecies)*
Muntiacinae: Muntjacs	Reeve's Muntjac *Muntiacus reevsi*	East China, introduced into UK *(2 subspecies)*
Hydropotinae: Water deer	Chinese water deer *Hydropotes inermis*	China, Korea, introduced into UK *(2 subspecies)*
Cervinae: Eurasian deer	Red deer *Cervus elaphus*	Europe, north Africa, Asia Minor, Tibet, Kashmir, Turkestan, Afghanistan, introduced into Australia and New Zealand *(12 subspecies)*
	Sika deer *Cervus nippon*	Japan, Taiwan, Vietnam, Manchuria, Korea, China, introduced into UK and New Zealand *(13 subspecies)*
	Wapiti *Cervus canadensis*	Western North America, eastern China, Manchuria, Mongolia, introduced into New Zealand *(13 subspecies)*
	Fallow deer ***Dama dama***	Widespread in Europe and Asia, introduced into Australia, New Zealand, Africa North and South America *(2 subspecies)*
Odocoilinae	**Roe deer** ***Capreolus capreolus***	Europe and Asia, north to Scandinavia, Siberia, east to China and Korea *(3 subspecies)*
	White-tailed deer *Odocoileus virginianus*	North and Central America, Canada, northern parts of South America, introduced into Scandinavia, New Zealand *(38 subspecies)*
	Black-tailed deer or Mule deer *Odocoileus hemionus*	Western North America, Central America *(11 subspecies)*
	Moose or Elk *Alces alces*	Northern Europe, Canada, northeastern America *(6 subspecies)*
	Reindeer or Caribou *Rangifer tarandus*	Scandinavia, European Russia, Greenland, Canada, Alaska, introduced onto arctic and antarctic islands *(9 subspecies)*

Dama dama dama (Linnaeus, 1758), the European (common) fallow deer and *D. d. mesopotamica* Brooke, the Persian fallow deer. The latter is now believed to be very close to extinction, however small numbers are kept in several zoos (Chapman and Putman, 1991). The European fallow deer is found in many parts of the world, despite its name (Table 1.1). It is currently the most widely distributed species of deer in Great Britain (Chapman and Chapman, 1975, 1980), although the fossil records suggest it has not been present continuously (Chapman and Chapman, 1975).

A detailed account of the history and fossil remains of the fallow deer is provided by Chapman and Chapman (1975). The best known fossil records of fallow deer that are similar to present day European fallow deer, are those of the Clacton deer (so called because of the localities in which the fossils were found). These fossils date from the second Interglacial period (Hoxnian), 250,000 years ago. These deer were larger than modern day fallow, similar in size to red deer (*Cervus elaphus*). However, remains of Clacton deer have not been found later than this period and were thought to have become extinct during the third (Riss) Glacial period, 180,000 - 130, 000 years ago (Chapman and Chapman, 1975). The fallow deer appears to have returned to Britain and was present in the last Interglacial (Ipswichian) period (100,000 years ago). Fossil remains of teeth from this time are intermediate in size between those of the present day fallow and the Clacton fallow. This suggests that deer of a slightly larger build than those seen today, were living in much of Europe approximately 100,000 years ago. A second extinction of this ancestral deer is believed to have occurred during the last (Würm) Glaciation (end of the Pleistocene epoch, 70,000 - 10,000 years ago), as no reliable remains have been found from Mesolithic and Neolithic sites in Britain

Following this extinction there has been much speculation as to how the fallow deer returned to Britain. If fallow deer had survived in parts of Southern Europe during the last Glacial period, they could have returned to Britain before it became an island, some 8,000 years ago. However, the lack of remains from Mesolithic and Neolithic sites suggests this was not the case.

The only alternative explanation is that fallow deer were reintroduced by man. The Gauls, Romans and Normans have all been suggested as candidates for this reintroduction (Chapman and Chapman, 1975). The only reliable evidence is from the eleventh century, when records report the hunting of 'dammas', which suggests that the Normans may have been responsible (Chapman and Chapman, 1975).

All the free-living fallow deer within Great Britain are thought to have descended from this medieval reintroduction or to be escapees from parks (Chapman and Putman, 1991). Ancient stocks are still believed to exist in Epping Forest (Essex), Savernake (Wiltshire), Rockingham (Northamptonshire) and the New Forest (Hampshire; Jackson, 1974). Fallow are the most popular captive species of deer found within the landscaped parks of British stately homes today (Chapman and Putman, 1991).

Description and Ecology

Fallow deer are medium sized, females measuring 60-70cm to the shoulder and males up to 90cm, females weigh 35-60kg and males 70-100kg (Putman, 1988). As a result of selective breeding of captive stock, escapees and reintroductions, there are many different colour varieties which range from white to black (Chapman and Chapman, 1975; Putman, 1988; Chapman and Putman, 1991). Typically however, in the summer their pelage is reddish fawn with white spots and a vertebral black stripe, in the winter they are greyish fawn with either indistinct or no spots (Putman, 1988, Chapman and Putman, 1991). The most discernable characteristic of fallow deer are the palmate antlers of the male, which are cast annually. Fallow deer are the only British deer to possess such antlers.

Fallow deer prefer deciduous or mixed woodland with an established understorey, although they have been known to occupy coniferous plantations (Chapman and Putman, 1991). They change their habitats throughout the year reflecting the seasonal availability of forage. The New Forest in Hampshire is one of the most favourable habitats for fallow deer (Section 2.2.1.1) covering some 375km^2 composed of deciduous and coniferous trees, grassland and heathland (Chapman and Chapman, 1980). The New Forest provided Jackson (1974) with a study site for the most comprehensive and detailed investigation to date into the feeding ecology of fallow deer. He found that there was a preference for deciduous woodland from September to March/April and for more open habitats, such as rides, clearings, pastures and arable fields from May to July.

In the New Forest, Jackson (1974) observed groups of 1-5 individuals, although groups of up to 70-100 animals have been noted (Chapman and Putman, 1991), usually when a number of small groups come together to share a favourite feeding place. Group sizes vary seasonally but males and females generally occur as discrete groups throughout most of the year. Males cast their antlers in April/May and by August/September their new set has grown and the velvet has been cleaned off. The males move into female ranges in the autumn (end of September / beginning of October) to breed (rut), they remain through November and December and then return to their own ranges (Chapman and Putman, 1991). The rut involves mature bucks competing to establish display areas or rutting stands to attract females. Females are pregnant from September/October to June/July while lactation continues through to the following March when most are likely pregnant again (Chapman and Putman, 1991). These different physiological states of males and females draw upon specific nutrients at different times of the year (Section 5.4.2).

Jackson (1974) found that fallow deer are preferential grazers (Section 1.2.2). Their diet consists of more than 60% grass March-September and up to 20% in winter (Jackson, 1974; Caldwell *et al*, 1983). The gut structure and physiology of these deer is adapted for this diet (Hofmann, 1988) and their morphophysiology will be discussed in Section 1.2.

1.1.2 Roe Deer

History and distribution

Roe deer belong to the genus *Capreolus* in the subfamily *Odocoileinae* (Table 1.1). There are two subspecies within this genus (Staines and Ratcliffe, 1991). The 'European' roe deer, *Capreolus capreolus* (Linnaeus 1758), is present in Europe and the Middle East, and *C. pygargus* (Pallas 1771), the Siberian roe deer, is found in Siberia.

Roe deer first appeared in the fossil record in the middle Pleistocene (Cromerian Interglacial, 500,000 years ago) in Europe and were absent from Great Britain during the Riss and Würm Glaciation periods when they were probably restricted to southern Europe (Lister, 1984). They were abundant in Great Britain during Roman and medieval times (Prior 1968). In the fourteenth century, the forest laws were changed, and as a result deer, including the roe, lost their protection and they went into decline with increased pressure from hunting and deforestation. Reintroduction into southern England and the Scottish lowlands took place at the beginning of the nineteenth century (Whitehead, 1964). Following this, deer numbers increased largely as a result of afforestation.

Description and Ecology

The European roe deer is relatively small (height: 60-70cm to shoulder, weight: 23-32kg) compared to most of the other deer present in Great Britain, the red deer (height: 105-140cm, weight: females 57-115kg, males 90-190kg), fallow deer (Section 1.1.1) and Japanese sika (*Cervus nippon*; height: 82-90cm, weight: females 35-40kg, males 70kg) are all larger, only the muntjac (*Muntiacus reevsii*) is smaller (height: 43-46cm, weight: 13-15kg; Prior, 1993). Roe deer show much less variation in coat colour than fallow deer, in summer they are reddish brown turning to a pale or olive-grey, greyish brown, to almost black in the winter (Staines and Ratcliffe, 1991).

Roe deer possess the remarkable feature of delayed implantation and are the only artiodactyl to show this (Staines and Ratcliffe, 1991). While the rut for most cervids takes place in the autumn, in the roe it occurs from mid-July to August. The blastocyst does not implant into the uterine wall until late December/early January (Short and Hay, 1966), with birth, most commonly twins, occurring between mid-May and mid-June (Staines and Ratcliffe, 1991). This allows both mating and birth to take place at the most favourable times of the year. The males cast their antlers from October to December, new ones grow and velvet is shed between March and April ready for the rut (Prior, 1993). As for the fallow deer, different physiological states of male and females roe deer require varying amounts of nutrients at different times of the year (Section 5.4.2). The males are territorial from April to August and the size of their territory is dependent on the habitat (Staines and Ratcliffe, 1991). Roe deer are usually solitary or occur in family groups (Prior, 1993), however in winter larger groups can be observed when they feed in open fields (Staines and Ratcliffe, 1991). Roe deer colonise young forest plantations and also mature woodland (Jackson, 1980), moorland (Henry, 1978) and upland forest (Cumming, 1966). They also exploit open habitats such as farmland (Zejda, 1978; Putman, 1986b).

A great deal of work has been done on roe deer forage and feeding behaviour, mostly in Eastern Europe (*e.g.* Poland: Siuda *et al*, 1969; Kaluzinski, 1982; Czech Republic: Holisova *et al*, 1986; Homolka and Heroldova, 1992; Hungary: Matrai and Kabai, 1989). In the UK, Hosey (1974) conducted a study on the diet and feeding ecology of the roe deer, Henry (1975) also looked at diet as did Loudon (1979) and Jackson (1980). These studies resulted in different accounts of the roe's diet throughout the year (Section 2.4.4.2). These variations could be due to differing geography, different habitat types and/or different methods of dietary analysis. The two analytical methods most widely used were rumen content analysis and faecal analysis (see Section 2.1.1).

Geography appears to play a large part in the different descriptions of the roe's diet. In Poland Siuda *et al* (1969) found 90 species of plants made up the roe's diet, whereas Helle (1980), working in Finland, found there were only 17 and 20% of these are not present in Britain or Poland (many were arboreal lichens). These geographic factors also apply within Britain. In the New Forest, Hampshire (Jackson, 1980) and Chedington, Dorset (Hosey, 1974) broadleaved species were the major food. However, in the Hamsterly Forest, Co. Durham (Henry, 1978), dwarf shrubs (*Calluna* sp. and *Vaccinium* sp.) were found to be important.

In the past, roe deer were known as 'browsers' (Prior, 1968) but this does not accurately explain their feeding behaviour as their diet is varied and can include many grasses. Using Hofmann's (1988) categorisation based on rumen morphology and size relative to body volume (see Section 1.2), roe deer have been re-categorised more recently as 'concentrate selectors' (animals that select plants or plant parts rich in easily digestible and highly nutritious cell contents). They have small mouthparts that facilitate selection of dietary items and a low rumen capacity in relation to body size. The food they ingest must therefore be highly nutritious and digestible, to ensure the flow rate through the relatively small rumen is fast enough to satisfy metabolic requirements. Roe deer must therefore be highly selective feeders, and according to Johnson (1984) the diet taken from a certain habitat, reflects selection and not availability. This being the case, it would be expected that roe deer would prefer high quality over low quality food. Although the specific factors influencing preference have not yet been identified, energy or nutrient or mineral content could be important.

1.2 DIGESTIVE PHYSIOLOGY AND METABOLISM IN RUMINANTS

1.2.1 Introduction

Herbivores are adapted to their diet in many ways. Many are ruminants, which have a complex stomach structure with four chambers. The chambers are the rumen, the reticulum (the rumen-reticulo complex), followed by the omasum and the abomasum. Food is ingested, goes into the rumen, is regurgitated back into the mouth where it is rechewed and reswallowed, this is a continuous cycle until the food is reduced sufficiently to enable it to pass into the reticulum, where it is then fermented. Regurgitation of the rumen contents and cessation of feeding are controlled by sensory cells in the muscle wall. Once in the reticulum, food is held in suspension with micro-organisms (bacteria, fungi) this also occurs in the rumen. Micro-organisms help digest the food material. The food remains in the reticulum until the cellulose of the plant cell walls has been digested by the micro-organisms. The process of fermentation produces volatile fatty acids (*e.g.* acetic acid) which are readily absorbed into the blood stream. The food material then passes into the omasum and then the abomasum, where digestion of fats, proteins, simple carbohydrates and other nutrients takes place. The products are absorbed through the wall of the small intestine. The presence of the microflora is the important factor of the ruminant stomach. They supply the animal with much of the energy and nutrients from plant cells by breaking down the cellulose cell walls. The regurgitation-chewing-reswallowing cycle aids the micro-organisms by increasing the surface area of the food available to them, by improving mixing and by increasing the duration of the first stage of the process thus giving more time for the micro-organisms to work.

1.2.2 Ruminant classification into feeding types

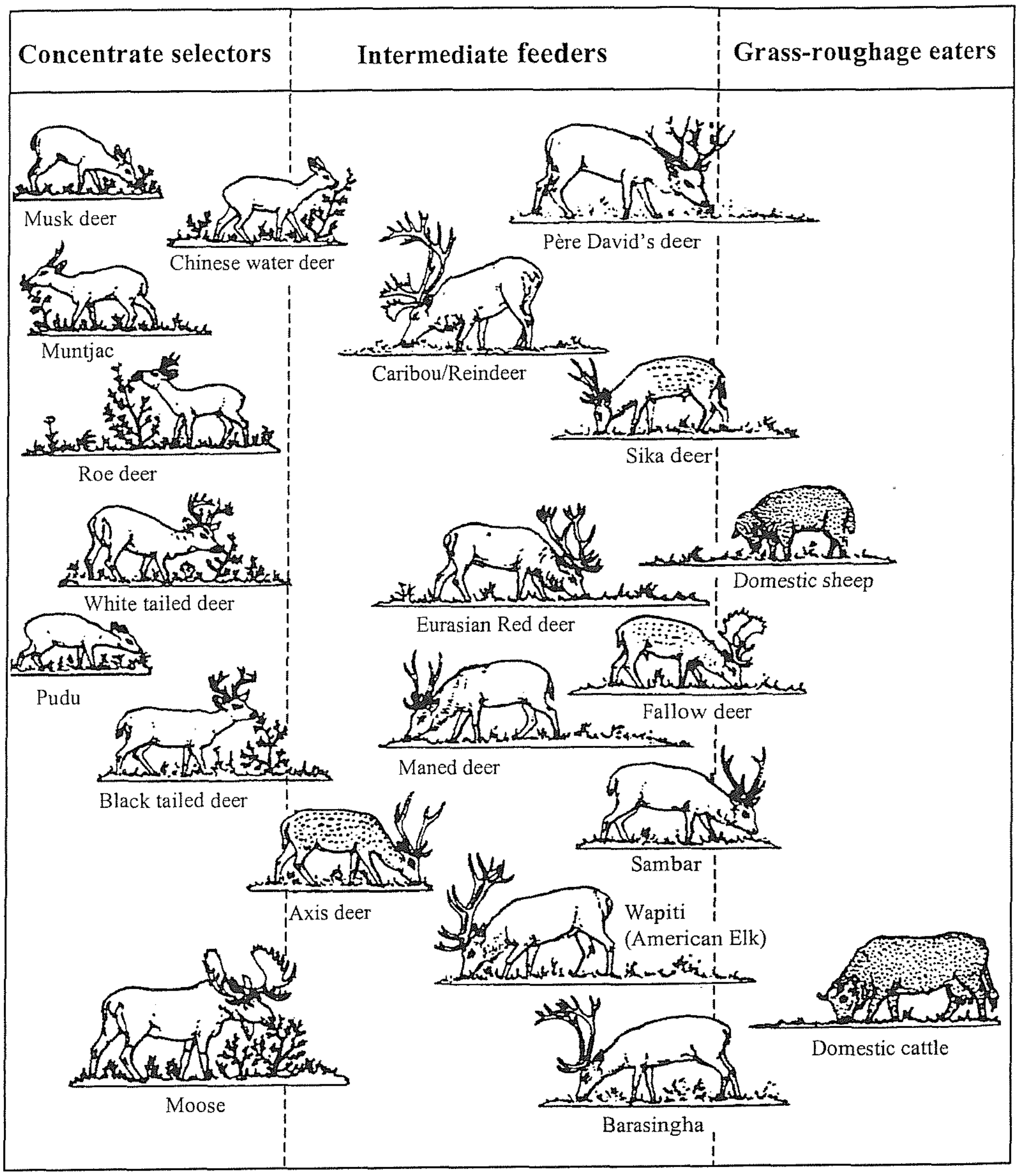
The driving factor determining the quantity and quality of food ingested is the need for energy (Kay *et al*, 1980). In ruminants this is related to the size of the animal and also its age and reproductive status. A small or productive ruminant must eat more digestible energy relative to its body weight than a large or non productive one. To achieve this, the animal must eat more or select a more digestible diet. A large or non productive ruminant can afford to be more flexible in its feeding habits because it can get enough energy from a relatively poor diet. Such an animal can adapt readily to a diet that changes seasonally in quality allowing it to be selective when better food is available (Kay *et al*, 1980).

Although the size of the animal can reflect what it eats, it is by no means a reliable indicator; as mentioned above, its age and reproductive condition are also important. It has been widely accepted for some time that the structure, development, relative size and importance of the different chambers within the stomach vary between species of ruminant according to their different diets (Hofmann, 1985, 1988). Hofmann (1988) states that all portions of the digestive tract show some degree of specialisation and adaptation. Anatomical variations that exist are the result of evolutionary trends in favour of specific food selectivity (Hofmann, 1988). These variations allowed Hofmann to classify ruminants, including the *Cervidae,* into three **morphophysiological feeding types** (Figure 1.1).

Ruminant species that evolved early and adapted to vegetation before grasses evolved and spread over the earth are known as **'concentrate selectors'**. These are animals that select plants or plant parts rich in easily digestible and highly nutritious cell contents such as starch, plant protein, fat and oil. They are, however, very limited in their ability to digest the cell walls which consist largely of structural components, cellulose and fibre. Deer that are classed as concentrate selectors include roe deer, white-tailed deer and mule deer.

Species of ruminant that evolved subsequently are more dependent on grasses and other fibrous plant material. Animals of this advanced feeding type are called **'grass and roughage eaters'** and include domestic cattle and sheep and many savannah antelope. The third feeding type is a heterogenous group known as the **'intermediate/mixed feeders'**. These are animals that show adaptations to either concentrate selectors or grass and roughage eaters. Members of this intermediate group can be very opportunistic feeders, they can be very adaptable seasonally and regionally. The most recognised deer in this category are red deer and fallow deer, although the latter tends to fall more towards the grass/roughage end of the category (Figure 1.1).

Figure 1.1 Classification of Ruminants, including *Cervidae*, based upon the three morphological feeding types. (Source: Hofmann, 1985)



1.2.3 Variation in ruminant anatomies

The division of ruminants into the three morphophysiological feeding types mentioned above, is based primarily upon the evolutionary adaptation of the digestive system. The principal anatomical structures that are specialised and adapted to specific feeding types are the salivary glands, the mouth, teeth, fore- and hind-gut.

Salivary glands

According to Hofmann (1988) all the salivary glands (buccal, mandibular, sublingual and parotid) reduce in size with increasing adaptation to plant wall digestion (*i.e.* more grass in the diet). Thus the parotid gland in the roe deer (concentrate selector) is 0.22% of its body weight, this falls to 0.08-0.1% for the red deer (intermediate feeder) and to 0.05% for the Mouflon (*Ovis musimon*: grass/roughage feeder).

However, these differences may be misleading, as an increase in salivary secretion occurs when ruminants and non ruminants are offered taninniferous food for sufficient time (Butler, 1989, as cited in van Soest 1994). Also, grazers do not possess the specific tannin-binding factors in their saliva (Robbins *et al*, 1991). Therefore, the comparison of the three feeding types is based on genetic differences between the species in addition to the adaptive effects of their respective diets.

Mouth parts and dentition

Mastication is vital for ruminant digestion as it reduces the size of the plant material thus allowing the ruminal microorganisms to work effectively. Mouth parts and teeth have adapted for the type of vegetation eaten and digestive strategy employed. Incisors and cheek teeth of concentrate selectors are more delicately built than roughage/grass feeders, and are more firmly attached in their sockets (Hofmann, 1988). Muzzle width and the hypsodontyl index have been measured in a number of ungulates (Janis and Ehrhardt, 1988) in order to investigate the hypothesis that grazers (grass/roughage eaters) have wider muzzles than the more selective species (concentrate selectors). Their data showed that concentrate selectors do have a tendency towards narrower muzzles which could also be associated with their longer tongues and prehensile lips that may aid in the food selection processes.

Foregut and hindgut

The foregut refers to the oesophagus and the stomach (rumen, reticulum, omasum and abomasum). The relative capacity and position of the four stomach compartments in the abdomen are characteristics of ruminants belonging to each feeding type. The surface of the rumen is covered by ruminal papillae, these are the sites of absorption. Their distribution, size and number are closely related to the feeding habits, forage availability and digestibility (Hofmann, 1988). Changes in papillae can occur in response to nutritional variation within the individual animal and are triggered by changes in the proportion of certain acids in the rumen. These adaptations can take two to three weeks to occur and are most pronounced in intermediate feeders. Papillae are also present in the reticulum.

The omasum consists of laminae which radiate inwards toward the centre of the chamber. The complexity and size of omasal laminae vary depending on the type of feeder. The microfauna found in the gut differs in the three different feeding categories. Roughage/grass feeders possess mainly cellulolytic bacteria specialised in breaking down cell walls whereas concentrate selectors have amylolytic bacteria specialised in breaking down readily digestible forage (Prins and Geelen, 1971).

The ratio of small intestine to large intestine also differs in the three different feeding types. Ruminants with a large subdivided rumen well suited to cellulolysis, such as roughage/grass feeders, have a much shorter proportion of large intestine to small intestine then those species which select for cell contents and readily digestible forage (Hofmann, 1988).

1.2.4 Study species - fallow and roe deer

According to the classification conceived by Hofmann (1988) roe deer are considered to be concentrate selectors generally having a simple gut structure. They have a small rumen, that is evenly covered in short papillae. The opening to the reticulum is wide and they possess an underdeveloped omasum and abomasum. This ruminal structure coupled with an overall short intestine means that the retention time (the time food remains in the whole gut) is short. All these structures in addition to the type of microorganisms present, are indicative of a concentrate selector's diet of easily digestible plants, primarily for the soluble cell contents (*i.e.* starch, plant protein, fat and oils). The structures favour less selective retention and increased passage of the carbohydrates and proteins.

Fallow deer are classed as intermediate feeders (Hofmann, 1988). These deer have physiological structures in between those of concentrate selectors and roughage/grass feeders, although they tend to be more inclined towards the latter category. As such they rely on their ability to digest cellulose which is reflected in their gut fauna. Compared to roe deer, fallow deer have a tendency to ingest relatively large amounts of plant material and retain it for longer in order to digest the food efficiently. This is reflected in the larger size of their gut structure and precedence of celluloytic gut microorganisms. The rumen has unevenly distributed papillae, more complex omasal laminae in the large omasum and a higher small intestine to large intestine ratio than in roe deer. Fallow deer appear to be more adapted to seasonal changes in availability and quality of forage (Hofmann, 1988).

1.3 DIET SELECTION

1.3.1 Introduction

This thesis was designed to investigate diet selection from two different approaches, firstly in the natural environment (Chapter 5) and secondly through controlled feeding trials (Chapter 6). These chapters will reveal if fallow and roe deer were selecting specific foods at certain times of year. They will then suggest whether or not this selection (if present) was based on the nutrient content of those foods when the availability of vegetation was taken into consideration. This section addresses the concept of diet selection by applying the explanations mentioned earlier: functional and causal or mechanistic, and more often than not a mixture of both. This will be done by considering several parameters involving the animal itself, the environment or niche the animal occupies and the characteristics of the food on offer. These variables can create many challenges, particularly for ruminants (Section 1.3.2). Five diet selection models are then considered that address these challenges (Section 1.3.3), the application of these in the current study (assuming diet selection is shown in fallow and roe deer) will be discussed.

1.3.2 Challenges faced by ruminants

Provenza and Balph (1990) believe that there are at least five problems or challenges that ruminants encounter when selecting individual plant items:-

Challenge 1) Variation among dietary items in the kind and amount of nutrient constituents
Different plant species and even the different parts of plants can differ greatly in the amounts of energy, protein and minerals that they contain (van Soest, 1994). Clearly an animal that could detect either the presence or absence of particular nutrients in their diets would be more successful than an animal that could not.

Challenge 2) Variation among dietary items in the kind and levels of chemical defences
Chemical defences of plants (secondary plant metabolites) can reduce digestion (*e.g.* cutin) or interfere with metabolic processes (*e.g.* tannins), some can even cause death (*e.g.* terpenoids; see Robbins *et al*, 1987a,b; van Soest, 1994). Fungal endophytes are also known to have detrimental effects after consumption (Durham and Tannenbaum, 1998). Some of these toxins can be detected by animals through taste, odour (Eisner and Grant, 1981) but others do not produce such signals

(Provenza *et al*, 1990). A successful ruminant would be one that can detect and avoid plant chemical defences, the question would be how do they do it?

Challenge 3) Plant morphological defences

Ruminants can be faced with plant physical defences, such as thorns or spines. Such morphological defences have been known to inhibit foraging or decrease the rate of digestion (Flores *et al*, 1989a,b,c). A productive ruminant will show clear preferences for plants, or plant parts, without such features or they will evolve ways of dealing with them.

Challenge 4) Temporal and spatial variation in the quantity and quality of forage

Animals including ruminants are faced with spatial variations in the quantity and quality of their dietary items, there can be variations within a plant and amongst plants of the same species (*e.g.* van Soest, 1994). Temporal variations also have to be considered; these can be within a day or a season for individual plant parts, and within a season or over generations for plant species. A successful ruminant would therefore be one that is in the right place at the right time to increase encounters with nutritional plants.

Challenge 5) Exposure of ruminants to unfamiliar foraging environments

Animals can be faced with an unfamiliar environment in which they have to forage, for example, when migrating or dispersing. A effective ruminant would be one that could adjust quickly in a novel environment.

1.3.3 Models of diet selection

The responses of ruminants to these challenges are described by Provenza and Balph (1990) in five diet selection models, which can be classed as being functional or mechanistic as described previously (Section 1.3.1):-

a) endogeneously generated hungers - **euphagia** (Section 1.3.3.1)
b) immediate sensory consequences - **hedyphagia** (Section 1.3.3.2)
c) body morpho-physiology and size (Sections 1.2 and 1.3.3.3)
d) learning through foraging consequences - **learning** (Section 1.3.3.4)
e) nutritional optimisation - **optimal foraging** (Section 1.3.3.5)

These five diet selection models will be considered individually in terms of the challenges laid out in Section 1.3.2 and their anticipated application to the current study. It is important to note that these models are not mutually exclusive and should not be considered as such. Where possible, ruminants are used as examples, but as much information is still limited for this group, other animals, especially laboratory rats, are also used as examples. There have been studies that suggest the selection mechanisms used by rats are similar to those used by some ruminants (reviewed by Provenza and Balph, 1987, 1988; Provenza *et al*, 1990).

1.3.3.1 Euphagia (endogeneously generated hungers)

Euphagia is a term for 'specific hungers' that are generated from within the body. This can only occur if an animal possesses an innate ability to detect the presence/absence of specific nutrients and toxins through taste and/or smell. The extent to which this may happen depends on a concept called 'nutritional wisdom', as suggested by Richter (1943) when working on rats.

The controlled feeding trials in this study were not designed to look into the existence of euphagia, this would require much more sophisticated and complicated trials which were simply not possible. Euphagia will be considered in the interpretation of the results of diet selection in the natural environment (Chapter 5) and from the feeding trials (Chapter 6). However, it can not be a realistic explanation as sodium, the one nutrient that animals are known to have a specific hunger for (see below), is not included in the chemical analysis. Sodium was not included as it is present in very small amounts in plant material (0.02-0.3%: Allen, 1989) and the spectrophotometer used was not sensitive enough to get reliable measures.

Euphagia does not include any associative learning (conditioning) between taste, smell or consequences after a food has been eaten (post-ingestive). The theory was developed by researchers from findings that livestock (see Arnold and Dudzinski, 1978) and rats (see Richter, 1943) select a more nutritious diet than would be expected if they had selected foods at random. This has also been used to suggest innate mechanisms for actively avoiding plants that contain secondary metabolites, *e.g.* monoterpenes (red deer: Elliott and Loudon, 1987).

If eupahagia exists, and there is still some doubt, then it could help to explain how ruminants recognise nutritional components of plants and how they deal with plant defences (*e.g.* toxins). In some cases what started out being explained by euphagia has been reinterpreted at a later date. Richter (1943) showed that when rats were presented with a variety of mineral sources they selected a balanced diet but also when deprived of one essential nutrient they could compensate by adding a new item to their diet. For example, rats that were deprived of yeast ate their own faeces to obtain the vitamin B complex. Richter (1943) tried to explain this behaviour by suggesting 'specific hungers' for each of the essential nutrients, however later work has shown that for rats this is not the mechanism of nutrient wisdom. Salt (sodium) and water are the only dietary components for which rats can detect a deficiency in their body tissue and select new diets to compensate for the deficiency (see Provenza and Balph, 1990).

Sodium specific hunger appears to be generated from within and is not reliant on experiences of sodium deprivation in the past. Ruminants such as sheep (Denton and Sabine, 1963) and cattle (Bell, 1984) have been shown to correct sodium deficiencies very accurately suggesting innate mechanisms for sodium hunger.

Apart from sodium no animal has been shown to have specific hungers for any other nutrient in the foods that they eat. Also, researchers have not demonstrated constant relationships between diet selection by ruminants and compounds such as protein, carbohydrates, sugars and organic acids (reviewed by Arnold and Hill, 1972). However, Provenza and Balph (1990) suggest that even if an animal (ruminant) can detect specific nutrients it does not necessarily mean that they innately recognise the nutritional benefit of eating such compounds.

Overall there is very little evidence for euphagia, other than for sodium. It is not anticipated that this model will explain how herbivores select or reject specific plants or plant parts. It must however be noted that very little research has investigated the ability of wild and domestic ruminants to detect the nutritional or toxic constituents of foods. The generally accepted rule is that animals compensate for deficiencies of essential nutrients by learning to avoid diets with adverse consequences instead of through specific appetites for the deficient nutrients (Rozin, 1976).

1.3.3.2 Hedyphagia (immediate sensory consequences)

Hedyphagia refers to the immediate sensory consequences that an animal receives when it first puts food into its mouth. This model came about as ecologists and nutritionists speculated that animals select plants or plant parts that are instantly ‘pleasing’ to their olfactory, gustatory or tactile senses, thereby obtaining a balanced diet and that they avoid vegetation that does not give them this sensation (Arnold and Dudzinski, 1978). If hedyphagia does exist, then through natural selection plants compounds that are nutritious would taste good and those that are toxic would taste bad.

As with euphagia, the feeding trials in this study were not conceived to look specifically at hedyphagia. However, the design of the preference tests, where some foods were intentionally made to be very sweet and therefore pleasing to the deer (Section 6.2.2), should mean that the data can be interpreted considering hedyphagia as a possible explanation in addition to other models.

Hedyphagia may help explain how, by association, animals obtain a nutritious diet and avoid plant chemical and morphological defences. Thus there is clearly some form of learning taking place (Section 1.3.7). Ruminants are known to select plant parts that are young, green and leafy, parts associated generally with nutritious forage, as opposed to those that are old, dead and tough (Provenza and Balph, 1990). They will generally avoid plants that have thorns or stinging hairs, that are difficult to ingest or are aversive to their tactile senses. Many researchers have found that many mammals will readily accept sweet tasting foods whilst rejecting bitter tasting ones, thus suggesting that sweetness is an indication of nutritious food and bitterness of toxic food (Bate-Smith 1972; Grill *et al*, 1984). Different species of ruminants in captivity have been shown to differ in their ability to discriminate and tolerate various purified sweet and bitter compounds (Arnold and Hill, 1972; Hofmann, 1989) and it is assumed that this also occurs in their natural environment.

Interactions between odour, taste and consequences of ingesting foods are not dealt with by the hedyphagia model, so it cannot explain why herbivores prefer to eat some plants that are toxic (Provenza *et al*, 1992) or avoid others that are initially 'pleasing' to the senses (Provenza *et al*, 1990). In these cases, there must also be some degree of learning taking place in the form of post-ingestive feedback (Section 1.3.7), *e.g.* avoidance learning. This has clearly been shown by many trials that have looked at conditioned flavour aversions and preferences. These suggest that palatability of food is relative and depends largely on the post-ingestive consequences (Mehiel and Bolles, 1988). As mentioned earlier, it is accepted that a sweet taste is an attractant whereas a bitter taste is aversive or a deterrent, however, Garcia and Holder (1985) have shown that preference for bitterness increases when paired with positive post-ingestive consequences and preference for sweetness decreases when paired with aversive post-ingestive consequences (*e.g.* nausea). It is suggested, therefore, that odour and taste may be important to ruminants for detecting subtle differences rather than for innately recognising plants that are nutritious or toxic. This recognition is based on positive or adverse gustatory and olfactory stimulation experience (Provenza and Balph, 1990).

There are many problems with applying avoidance learning to herbivores. The meals that they eat are not discrete, especially for those herbivores with multiple stomachs and the meals rarely contain a single food type. Adverse consequences of eating often develop over a long period and the effects of many hours or days of eating might be confounded. Also, as stated many plants have adverse consequences but are nevertheless an essential component of a herbivore's diet.

Evidence has supported the view that generalist herbivores are limited in their ability to select food using avoidance learning. For example, sheep and donkeys (*Equus asinus*) cannot avoid food with adverse consequences if the delay is long or if they eat a mixed meal (Zohorik and Houpt, 1981). This may suggest that herbivores do not possess sophisticated 'nutritional wisdom' (Arnold, 1981).

When faced with a novel food an animal will respond based upon its previous experiences of specific food flavours (taste, odour) and also the post-ingestive consequences of those flavours despite the possibility that the novel food may be very different in this respect (Provenza and Balph, 1987, 1988). Hedyphagia therefore, is similar to euphagia in that it cannot stand alone as a model of diet selection but is closely intertwined with mechanisms associated with learning.

1.3.3.3 Body morphophysiology and size

There can be many constraints that limit an animal's ability to recognise, select and utilise a particular food from that available. These constraints are both intrinsic and extrinsic. For ungulates **intrinsic** constraints are related to the animal's body size, morphology, physiology and psychology or behaviour (*e.g.* perception and discrimination). These constraints interact with the **extrinsic** constraints of the environment, *i.e.* the nature of the food source (*e.g.* morphology, toxicity). Both intrinsic and extrinsic constraints will be considered when interpreting the data (Section 7.2.3), particularly as fallow and roe deer are classified as different types of feeders (Section 1.2.2).

For herbivores there are four morphological parameters that may affect forage selection (Hanley, 1982). These parameters are body size, the type of digestive system (cecal or ruminant), the rumino-reticular volume to body weight ratio and mouth size. By evaluating these parameters it is possible to determine the types of food a specific herbivore can exploit most efficiently. Body weight and type of digestive system determine the overall time-energy constraints within which the ungulate must secure its food. The rumino-reticular volume determines the type of food items that the rumen is most efficient at processing. Mouth size is important in determining the degree of selectivity that is mechanically possible and is highly correlated with feeding style (Gordon and Illius, 1988). Animals with small mouths, for instance, are capable of selecting specific plant parts (Jarman, 1974; see section 1.2.3).

Two models have been developed for understanding diet selection based on differences in morphology (Provenza and Balph, 1990). The first of these models classifies ruminants into concentrate selectors, intermediate feeders and grass-roughage feeders (Hofmann, 1988, 1989). This has been addressed in Section 1.2 and will not be considered again here.

The second model investigates the relationship between body size and metabolic requirements (Bell, 1971; Jarman, 1974; Demment and van Soest, 1985). The body size of an animal has implications on the minimum quantity and quality of food necessary for survival and thus the areas selected for foraging. Bell (1970) and Jarman (1974), investigated the allometric scaling of metabolic rate in ruminants, which became the Bell-Jarman principle. They argued that small antelope (small ruminants) require high quality (low fibre) food to satisfy their high metabolic rates. Small ruminants have higher metabolic costs per unit volume of rumen than larger ruminants. Therefore, the forage material these small animals select must produce higher fermentation rates and energy yields and increased throughput than that selected by larger ruminants. Increased throughput results in lower digestion of cell walls by small ruminants, the higher the ratio of rumen volume to body weight the more cell wall can be tolerated in the diet. This principle was supported by the work of Demment and van Soest (1985). They claimed that large ruminants had a greater capacity to process and survive on poorer quality foods which produce slow fermentation rates.

From this a model of digestion kinetics was developed to predict the intake and digestion of any specified forage by ruminants and hind gut fermenting species (*e.g.* equids; Illius and Gordon, 1991, 1992). Gordon and Illius (1988) found that the quality of grass which was adequate for grazers can provide about 75% of the a browser's requirements, this demonstrated the importance of body size for diet selection, as browsers are invariably smaller than grazers. Highly digestible grasses, *e.g.* fresh spring growth, would be sufficient for the smallest animals at this time and followed by a shift to more lucrative forage at other times of the year (Gordon and Illius, 1988).

Conversely a large herbivore could obtain their requirements from eating forbs (broadleaved non woody herb) however they would have to eat a great many of these as they are generally not ubiquitous (unlike grass), so they have to be selected individually. Grazing animals have dental morphology unsuited to this kind of selectivity, their incisors are broad and flat, whereas browsers have a narrower and more pointed incisor arcade capable of greater selectivity (Gordon and Illius, 1988). Gordon (1986) estimates that a small browser would have to take in 15,000 plant items per day to meet their energy needs and that this would be only a fraction of the needs of a larger animal.

From this it becomes apparent that the distribution of food and the potential digestibility broadly determines the diets that animals of different body size must select (Illius and Gordon, 1993). Illius and Gordon (1993) state that animals have evolved to exploit plants within subsets of quantity-quality ranges and expect them to be able to recognise their feeding niche as the quantity-quality area where diet selection will be least constrained.

The two models referred to in this section, morphophysiology and size, operate through animals sampling plants. These models make general predictions about foraging in unfamiliar environments and in environments where the abundance and quality of the forage fluctuate temporally and spatially (see Illius and Gordon, 1993). Larger ruminants tend to forage over more habitats, where available, due to their tolerance for a wider variety of plant species.

Although these two models help to understand diet selection they do not explain it in quantitative taxonomic terms (Hanley, 1982) because of the complex chemical and morphological characteristics of plants, and also because they do not take consider the behaviour of the animal (Provenza and Balph, 1988; Provenza *et al*, 1990).

Provenza and Balph (1990) believe that explaining diet and habitat selection only in terms of body morphophysiology and size fails to take into account intraspecific variation and plasticity. Genetic variation coupled with environmental influences produce different individuals which in turn select different habitats and diets (Provenza and Balph, 1987, 1988). For example, animals considered to be grazers, such as bison (*Bison bison*) and cattle often prefer and are more productive on ranges that are dominated by shrubs (*e.g.* Provenza *et al*, 1983; Waggoner and Hinkes, 1986). The same is also true for some animals that are regarded as browsers, such as mule deer; these animals often prefer and do well on grass diets (*e.g.* Urness *et al*, 1983). Provenza *et al* (1990) have also identified individual variation amongst goats in terms of their tolerance of blackbrush twigs that are high in tannins.

1.3.3.4 Learning

An animal learns through consequence. It is assumed that the evolution and modification of diets is a result of positive and negative consequences that have occurred from foraging (Provenza and Balph, 1990). This will be addressed in this study through the interpretation of the results from the feeding trials (Section 6.4). The preference tests have been designed to highlight the possibility of any learning taking place.

A herbivore has very little information about a plant before it takes a bite. An animal that is naive of the particular food item (*i.e.* it has never encountered it before), may accept or reject it based only on an innate response. Alternatively they may receive visual, olfactory and tactile stimuli which they could relate to other, similar forages they have eaten before and so make a more informed decision. Experienced feeders are those animals that have experienced and learnt about aversive and positive stimuli and reject or accept forage accordingly. The stimuli could be pre-ingestive (tactile, olfactory, visual or gustatory) or post-ingestive (nutrient absorption providing essential feedback; Provenza and Balph, 1990). Animals can, in some situations, learn from only one exposure to a particular forage whereas for some plant species rejection occurs only after several experiences. A herbivore's perception of nutritive value is generally not immediate, most nutrients cannot be recognised on consumption (Arnold, 1981), more likely after absorption which could be several hours later (Provenza and Balph, 1990). Roe deer fawns learn through negative consequences of consumption and are able to recognise aversive foods by smell alone (Tixier *et al*, 1998).

The learning model, according to Provenza and Balph (1990) is flexible despite being mechanistic and is based upon the assumption that the inherent ability to learn is the result of natural selection in changing foraging environments. Until recently learning was not a concept that was considered to be an explanation for diet selection, but now several researchers have looked to psychology for greater understanding (*e.g.* Zahorik and Houpt, 1981; Provenza and Balph, 1987, 1988).

The five foraging challenges that ruminants face listed in Section 1.3.1 are all addressed by the learning model: pre-ingestive experiences are associated with a plant's physical defences, temporal and spatial variation, and unfamiliar forage environments; post-ingestive experiences are primarily associated with variations in nutritional content and chemical defences of plant species and plant parts.

The most extensive area that has been studied regarding learning is concerned with associating specific foods with aversive post-ingestive consequences (*e.g.* Provenza and Balph, 1987; Provenza *et al*, 1990). This is known as conditioned food aversion and an animal learns to avoid a particular food by associating its taste or smell with the nauseous feedback it previously experienced (Garcia and Holder, 1985).

A shift in the palatability of a food to an animal through conditioned learning, is caused by stimulation of the emetic system of the midbrain and brainstem (Garcia, 1989 cited in Provenza and Balph, 1990). Any physiological, emotional or perceptual event, or any physiochemical agent that causes nausea, can create a conditioned food aversion. These do not even have to cause obvious signs of nausea, provided that it affects the emetic system (Garcia, 1989 cited in Provenza and Balph, 1990).

According to Provenza *et al* (1990) ruminants may show some difficulty in learning to avoid foods that contain toxins that do not affect the emetic system, *e.g.* strychnine and cyanide. In rats, chemicals that affect the nervous system and metabolic processes, cause neuromuscular blockage or block pain and create strong place avoidance behaviour but only weak food aversions (Lett, 1985; Garcia, 1989 cited in Provenza and Balph, 1990). Also in rats, bloating, allergies and lower intestinal discomfort have been shown not to instigate food aversions (Pelchat *et al*, 1983; Garcia 1989 reported in Provenza and Balph, 1990). On the other hand, lithium chloride is a chemical that causes nausea in many animals (Prien *et al*, 1971) and, in mammals it results in strong food aversions.

A great deal of research has been carried out primarily on rats, into their ability to associate foods with post-ingestive consequences. This research has clearly indicated that rats form strong preferences for:-

a) non nutritive flavours paired with calories (Bolles *et al*, 1981; Messier and White, 1984; Mehiel and Bolles, 1984)

b) foods paired with recovery from nutritional deficiency (Zahorik *et al*, 1974; Baker et al 1987; Baker and Booth, 1989b)

c) foods paired with recovery from post-ingestive distress (Green and Garcia, 1971; Sherman *et al*, 1983)

Provenza and Balph (1987) suggest that these findings can be extrapolated to ruminants and have been investigating these preferences in ruminants for many years, their findings are very encouraging. Sheep form strong preferences for non-nutritive flavours paired with calories (Burritt and Provenza, 1992). Also, Provenza *et al* (1983) found that cattle develop a preference for supplemental protein blocks when on low protein forage.

One of the main differences between ruminants and rats is their digestive system. Many byproducts result from microbial fermentation that occurs during ruminal digestion, such as volatile fatty acids (VFAs) and ammonia. It is highly likely that these byproducts could produce conditioned food preferences. Other substances, according to Provenza and Balph (1990), that could have similar effects are pancreatic hormones (*e.g.* insulin) and brain-gut hormones (*e.g.* cholecystokinin). In ruminants there are changes in portal blood metabolites, such as VFAs and insulin, within 15 minutes of the start of a meal (Chase *et al*, 1976). These changes allow ruminants to associate specific foods with their post-ingestive consequences very quickly, and to form strong preferences or aversions. It is not believed that a single nutrient, compound or hormone will have a dominant role in producing conditioned food preferences, given the variety of nutrients animals need, many different mechanisms will be involved (Provenza and Balph, 1990).

Provenza and Balph (1990) categorise pre-ingestive experiences of animals into four different types: *in utero*, via the mother's milk, through social means and through trial and error. It is their belief that these experiences together with many post-ingestive consequences, form the diets of ruminants.

a) *In utero* - whilst in the uterus a foetus can experience many compounds in plants that cross over from the mother via the placenta. Rats have been shown to prefer and avoid specific food flavours based on experiences acquired *in utero* (Stickrod *et al*, 1982; Smotherman, 1982; Hepper, 1988). It has been suggested by Hill and Przekop (1988) that there may be critical periods when learning can take place whilst *in utero*. Bradley and Mistretta (1973) have demonstrated that foetal lambs have the sense of taste during the third trimester of gestation. Others have hypothesised that in ruminants such as goats, sheep and cattle, taste experiences as a foetus affect adult taste preferences (Bradley and Mistretta, 1973; Provenza and Balph, 1988). Tixier *et al* (1998) showed spontaneous selectivity in roe deer fawns and considered that although unlikely, learning could have taken place *in utero*.

b) **Mother's milk** - the flavour of a mother's milk can be affected by the foods that she eats (Bassette *et al*, 1986). In rats (Galef and Sherry, 1973; Capretta and Rawls, 1974) and ruminants (Nolte *et al*, 1990) this has been shown to affect later preferences for solid food, offspring preferring to eat what their mother did.

c) **Social learning** - when a young animal moves onto solids and starts to forage, the experience of its conspecifics will play a role in what it selects to eat (*e.g.* Provenza and Balph, 1991). The most simple role model is the mother, a youngster will 'copy' its mother, eating and avoiding the same foods as her (see Provenza and Balph, 1987, 1988; Thorhallsdottir *et al*, 1987). Rats have demonstrated that they can learn about their foraging environment through olfactory cues from conspecifics (Galef and Heiber, 1976; Galef and Wigmore, 1983; Galef *et al*, 1984). Although there has been no evidence for this in ruminants, olfaction does play an important role in their communication. Through olfaction they can identify previously absent individuals, their physiological state and how long ago they were present (see MacDonald, 1995). It is possible, therefore, that a ruminant could use information from a conspecific to determine which locations they fed at and which plants they ate thereby facilitating their own selection.

d) Trial and error - when a young animal begins to forage alone it then starts to also learn by trial and error. In order to do this, it has to be able to remember what it has eaten and to sample novel foods with caution (for review see Rozin 1976). Young ruminants have been shown to remember specific foods that produced a negative (Burritt and Provenza, 1991) or a positive consequence (Squibb *et al,* 1990), for at least one to three years (see Provenza and Balph, 1987, 1988). They have also been observed to sample new foods cautiously (Provenza *et al*, 1990; Thorhallsdottir *et al*, 1990; Burritt and Provenza, 1991). When an animal experiences either an aversive or a positive post-ingestive consequence it should associate the response to novel and not familiar foods (Provenza and Balph, 1990). Provenza and his team found this to be the case with goats and sheep in conditioned food aversion experiments (Provenza *et al*, 1990; Burritt and Provenza, 1991).

Sampling and resampling foods is very important particularly for herbivores, as even familiar foods can change in nutrient and toxin concentrations over a short period of time (Freeland and Janzen, 1974; Westoby, 1974, 1978). Sampling means they ingest a wide array of food which in turn reduces their chances of over-ingesting toxins and increases their chance of meeting their full nutritional requirements. It also means that they can detect changes in toxicity and allows them to find new foods. The extent to which ruminants discriminate and generalise depends largely on the similarity among different plant species and parts as determined by odour, taste and post-ingestive consequences, and the specific animal's sensory abilities (olfaction, gustation and visual; Provenza and Balph, 1990). In these terms what presents itself as a novel food to a ruminant may be grouped with others of a similar smell or taste, for example, which may or may not reflect the conventional plant taxonomic classification system based on families and species.

Some ruminants may be faced with foraging in an unfamiliar environment, *e.g.* through dispersal. In these circumstances the animal will rely on the similarity between the new environment and their old familiar one. The greater the difference the harder it will be to forage and find shelter etc.

This learning hypothesis is viewed particularly by Provenza and Balph (1990) to have a significant contribution to diet selection by ruminants. It is thought to provide "a mechanistic framework in which to study the acquisition of dietary habits, foraging skills and habitat selection patterns by ruminants".

1.3.3.5 Optimal foraging (nutritional optimisation)

This section will first outline the basis of optimal foraging theory. Then concentrate on how it applies to herbivores and finally to large herbivores including the ungulates. The current study is not attempting to use optimal foraging theory to explain the data, but it is believed that an understanding of the concepts and beliefs is useful in the overall interpretation.

An animal is faced with a number of competing demands throughout its life, such as avoiding predation, securing a mate etc. Evolutionary pressures demand that the animal carries out these functions in an optimal way. Animals have evolved abilities to achieve optimal solutions to the problems they encounter over their lifetime. It is assumed that an animal behaves in an optimal way, however it may not be obvious what it seeks to optimise or what rules it employs. Optimality models have been created that make quantitative predictions of how an animal will behave in a given set of circumstances. By observing the animal's actual behaviour under such conditions it is then possible to accept or refute each of the models proposed.

Foraging behaviour has been investigated and analysed extensively with the use of optimization models (see Stephens and Krebs, 1986). These models do not determine if an animal is foraging optimally but rather by what rules it forages.

Optimal foraging problems that an animal is likely to encounter are usually those concerned with choice. For example, an animal that feeds in a 'patchy' environment (food distributed irregularly) has to make the choice of when to leave one patch for another. For choices to be made a number of factors must be considered; time and effort to travel between patches; overall prey density in the animal's environment; time and energy needed to capture and consume particular prey types. For optimal foraging an animal must consider these factors and act upon them. It would therefore be beneficial for the animal to have some learning processes and memory operating (McNamara and Houston, 1987).

Mechanisms for foraging optimally are not clearly understood and are more than likely different for different species. The mechanisms may be innate or, learned and stored in memory, or both. Animals may have mechanisms for remaining in a relatively rich patch which rely very little on learning abilities. Some animals show 'area- restricted' search: they encounter a prey item, then decrease their travelling speed or increase the intensity of their searching, so insuring that they stay roughly in the same area. This implies that the more frequent the animal encounters prey the longer it will stay in that patch. This could involve some learning, but equally it could be a preprogrammed response tendency, 'switched on' when a prey item is found.

Models have shown that optimal foraging requires that the animal processes and stores relatively large amounts of data (Kamil and Roitblat, 1985). If learning mechanisms are involved in optimal performance, there is a need to recognise profitable and unprofitable prey, discriminate rich patches from poor, gather and store information on the density of prey types, etc.

Hughes (1993) summarises the fundamental assumptions from the basic optimal diet models, a forager:-

a) can evaluate the profitability, in terms of yield per unit handling time, of each food type encountered and rank this relative to the profitability of other types;

b) can eat and remember the average profitability of all food types encountered, that the forager can measure encounter rates with different food types;

c) uses all this information to decide which encountered items to accept and which to reject.

He points out that the two strongest predictions are that a forager should always accept the most profitable food type and that it should accept successively less profitable types only when encounter rates with higher-ranking types fall below critical levels. Hughes (1993) concludes his summary with the opinion that "... the diet therefore should expand and contract according to the quality and availability of alternative foods".

Although Hughes (1993) was talking about all animals in his summary, this latter statement appears in principle to be related to herbivore foraging behaviour and so attention is turned specifically to herbivores.

Optimal foraging in herbivores

Carnivore diets generally consist of prey with approximately the right balance of nutrients and with little variation between them, so rate-maximising models (*e.g.* rate of energy intake) can be used for these animals. However, for herbivores it is very different, especially for generalist herbivores. These animals feed on abundant but low quality prey and have the problem of selecting a balanced diet as well as maximising their energy gain (Crawley, 1983). Also, in addition to these problems many plants contain toxic substances.

There are conflicting views with regard to the models used for herbivores, some believe that the rate-maximising models may still be useful in interpreting herbivory (Stephens and Krebs, 1986). To help unravel the complexity of herbivore diet selection the following points must be addressed.

a) herbivores do not spend much of their time searching for food, the majority is spent ingesting and digesting the food, therefore time budgets differ from those of carnivores.

b) herbivore food does not usually come as neatly packaged prey items, so the concept of 'encounters', as used for carnivores, is not the same.

c) for some small herbivores the diet consists of only one plant species throughout their whole life (*e.g.* caterpillars), these animals do however have to make choices as to which part of the plant to feed on. Choices are also made by larger herbivores (*e.g.* ruminants, Provenza and Balph, 1990) between plant parts.

Therefore the decisions and assumptions that have been made for models concerned with 'typical predators' cannot be the same for models concerned with herbivore foraging. Belovsky (1978, 1981, 1984) has suggested that so-called 'prey choice' in typical predators may, for herbivores, be a decision about diet composition rather than dealing with acceptance or rejection following an encounter with food.

Stephens and Krebs (1986) have reviewed the literature concerning herbivore foraging and have arrived at three views about the nature of decision, constraint and currency for herbivore diets:

1. *Rate- or amount-maximising subject to constraints*

This assumes that herbivores maximise the rate of energy intake, (or energy gains over a fixed time - Belovsky 1978) or nitrogen (Owen-Smith and Novellie, 1982) or maximize digestion rate (Westoby,

1974) if digestion is slower than ingestion (*e.g.* in ruminants). But, they also take into account requirements for one or a small number of essential nutrients, such as sodium (Belovsky, 1978) or the avoidance of poisons, which constrains their rate-maximising.

Stephens and Krebs (1986) give three observations offering evidence for the rate-maximizing view. Firstly the concentration and availability of different nutrients in plant species (or parts) are correlated. Highly digestible plant parts also tend to be high in protein (Arnold, 1981), also the general pattern for most plants is that young leaves are richer in all amino acids than older ones (Glander, 1981). Therefore by selecting certain plants or plant parts to maximize the intake of one nutrient, the intake of many or all nutrients may be maximized at the same time. Secondly, herbivores do not appear to be able to detect specific nutrients, except for sodium and water (see Section 1.3.4). This factor makes complicated models where diet choice interacts with nutrient limitations, less likely. The third observation lending credibility to the view is that many herbivores, including ruminants, can make most amino acids and many other essential nutrients (Owen-Smith and Novellie, 1982) and so many do not need a complex diet to meet their requirements.

The most well known research in this particular area is by Belovsky working on the diet of the moose (*Alces alces*). In 1978, he identified sodium as being a nutrient constraint for several reasons.

1) vertebrates need large amounts of sodium, as they must replace that lost each day;

2) sodium together with water are the only nutrients for which there 'specific hunger' (euphagia; see Section 1.3.4);

3) sodium is often scarce in plants (0.02-0.3%, Allen, 1989).

Belovsky (1978) hypothesised that the moose maximises its daily energy intake subject to a sodium constraint. The moose chooses any mixture of high sodium, low energy aquatic plants and low sodium, high energy terrestrial plants. The daily sodium and energy requirements were estimated, as were the constraints imposed by rumen size. It was shown that the proportion of aquatic plants in the diet was very close to that predicted by the energy-maximizing linear programming model. However, the linear programme failed to predict the moose's choice of individual species within the terrestrial habitat (Belovsky, 1981). This may have been due to the presence of toxins. Belovsky's linear programming models have been widely criticised (*e.g.* Hobbs, 1990). However, Belovsky has extended the models to other generalist herbivores (*e.g.* Meadow vole, *Microtus pensylvannicus*:

choice between grasses and forbs and the Kudu, *Tragelaphus strepsiceros*: choice between herbs and shrubs) and actively defends his findings, although admits there are serious limitations (Stephens and Krebs, 1986).

2. *Selecting complementary nutrients*

Rapport (1980) has reported that the best way to view herbivores is not as maximising the rate of intake subject to constraints but as choosing between packages that consist of complementary mixtures of dietary components. These complements could be nutrients or inhibitive poisons (Levander and Morris, 1970; Freeland and Janzen, 1974).

Rapport supports the 'complementary view' by his own study on protozoa (*Stentor*), showing that they grew better on diets of natural prey than on pure diets. This has also been shown for other animals, *e.g.* insectivorous birds (Krebs and Avery, 1984). Rapport also states that *Stentor* chose a mixed diet when more than one prey type was available, and that preference for a particular prey species increased as a function of its abundance. However, Stephens and Krebs (1986) argue that these results do not give enough evidence for the complementary model and could quite easily be explained by the constraints model mentioned earlier. There is, in fact, very little evidence for this model of herbivore foraging.

3. *Avoiding toxins*

Diet choice in herbivores is thought by many to be determined by plant toxins (Freeland and Janzen 1974; Harborne, 1994). Rhoades and Cates (1976) were one of the first groups of people to discover that there are two types of chemical defence for plants, firstly chemicals such as tannins that lower the digestibility of plant proteins to all herbivores when in sufficient concentration. Secondly there are other chemicals that are more poisonous, such as cyanogens and cardenoids; these work at low doses but are often overcome by specialist herbivores. The tannin-like toxins can be considered using the rate- or amount-maximising models, as mentioned earlier. The more poisonous toxins may limit the range of plants types available to a herbivores (Stephens and Krebs, 1986). Some people believe that the more poisonous toxins strongly influence diet choice for large generalist herbivores (*e.g.* Bryant and Kuropat, 1980), whereas others believe that nutritional quality and the presence of tannins are more important (*e.g.* Arnold, 1981; Glander, 1981).

For herbivores it is generally agreed that plant qualities other than energy are important in diet selection (Freeland and Janzen, 1974; Milton, 1979; Rapport, 1980; Owen-Smith and Novellie, 1982). In this case the simple energy maximising model is unlikely to be adequate.

From a review of the literature by Stephens and Krebs (1986), it seems apparent that selecting complementary nutrients does not, at this point, have any evidence to reinforce it and although avoiding toxins is very important it is unlikely to be the main parameter for diet choice. So, the evidence points to the rate-maximising models with constraints being the explanatory tool for herbivores in general. This, as mentioned, also takes into account toxins and poisons but as one of many constraints. However, having tentatively settled on this view of herbivore foraging there are still many problems yet to overcome.

Optimal foraging in large herbivores and ungulates

Work on herbivore foraging strategies has been carried out by a number of people. According to Hanley (1982), to apply the existing models of optimal foraging certain assumptions must be made: generalist herbivores can rank potential food items according to net value; the animals keep track of constantly changing values of food items and adjust their ranked values as a function of differing phenologies and habitats; foraging ungulates must detect values of individual species while consuming continuous multi-species meals; ruminating herbivores have an added problem of different nutritional value of a food item as a consequence of other food items in the rumen (Church, 1975 cited in Hanley, 1982). Westoby (1978), considering equines, ungulates and lagomorphs, says that these optimal foraging assumptions are not met by large generalist herbivores. It is, however, possible to predict the general diet type that an ungulate would select if it was given the choice.

The problems that surround herbivores have been outlined by Owen-Smith and Novellie (1982), and are reasons why it is difficult to apply existing optimal foraging strategies. They state that herbivores are usually surrounded by a surplus of potential food items, so that pursuit time and relative catchability are irrelevant. Also plant foliage is generally low in nutritive value making it necessary to ingest bulk amounts, this makes eating times and digestive processes important constraints. Finally, different plant species and parts of plants can vary widely in their nutrient contents, so it is important that the animals control the quality rankings of potential foods, as mentioned in the assumptions above (Hanley, 1982).

According to Hanley (1982) this framework is not sufficient to predict the species composition of optimal diets. However, it does give more of an insight into the competition and resource partitioning in ungulate communities than has been provided by current models of optimal foraging strategies.

One of the main problems with foraging models highlighted by Illius and Gordon (1993) is that the structural complexity of plants must be considered; plants vary greatly in their composition and digestibility. They go on to say that plant quality should be considered as a variable with great ability to affect choice.

Rate maximising models are used for mammalian herbivores (Belovsky, 1978; Owen-Smith and Novellie, 1982) but there are a number of reasons for questioning whether energy maximisation applies to vertebrate herbivores (Crawley, 1983). Westoby (1974), for example, argues that for herbivores maximising energy intake may be less important in diet selection than obtaining a balanced diet and avoiding toxins and other anti-nutritional plant compounds. However, there is very little evidence to suggest that herbivores select nutrients to balance their diets and particular dietary or anti-nutrient factors can be incorporated into rate-maximising models by specifying constraints (Stephens and Krebs, 1986).

The main problem facing such models is the complexity of vegetation structure and composition, with the variation in nutrient content requiring mixed diets to balance nutrients. The major source of variation in nutritional content of vegetation is the presence of lignocellulose in maturing plant tissues, the digestible fraction shows much less variation (Illius and Gordon, 1993). As a result foraging strategies that aim for both energy and nutrient maximisation are likely to be achieved by tactics that maximise the intake rate of digestible plant tissues. Therefore rate maximisation appears to be the most likely explanation for large herbivore foraging behaviour subject to the avoidance of harmful chemical compounds and constrained by inherently difficult task of discrimination between foods on the basis of their absolute nutritional yield (Illius and Gordon, 1993).

1.3.3.6 Summary

Three of the models (learning, and to some extent morphophysiology and optimal foraging), address all the challenges a ruminant faces, however it is evident that there is a lot of crossover between the models and they should not be considered separately. Provenza and Balph (1990) state that the morphophysiology and learning models are complementary in that behaviour (learning) is a result of consequences determined by interactions between neurological, morphological and physiological processes. It should be noted that learning is present within all the models. For an animal to be successful it has to have some ability to learn and remember.

Illius and Gordon (1993) have carried out much of their work on looking specifically at ungulates.

From their work on red deer they have been able to say that the mechanisms of diet selection are the function of two vegetation properties, quantity and quality, that interact with the animal's size through processes regulating food intake and digestion (Illius and Gordon, 1987, 1991).

This thesis addresses the mechanisms of diet (nutrient) selection by looking at the quantity and quality of vegetation eaten by two morphologically and physiologically different deer, fallow and roe deer. It also investigates experimentally the mechanism of learning that is inextricably linked to diet selection.

1.4 AIMS AND RATIONALE

The main question of this research was

Do fallow and roe deer select specific foods based upon the nutrient content?

This question was addressed in two ways. Firstly by looking at the quantity (Chapter 2), availability (Chapter 3) and quality (Chapter 4) of vegetation eaten over one year, to uncover trends between nutrient content and the amounts eaten of specific plants (Chapter 5). Secondly by conducting a series of feeding trials differing in one particular nutrient (protein; Chapter 6).

If deer appear to select specific foods based upon the nutrient content then there must be underlying mechanisms or strategies involved in the selection process as described in Section 1.3. This then leads on to the following research question:

Can diet selection by deer be attributed to euphagia, hedyphagia, morphology, learning and/or optimal foraging?

This question will be answered the General Discussion (Chapter 7) by applying the data generated from the observational part of the study (Chapter 5) and from the feeding trials (experimental) to the models described in Section 1.3.

Fallow and roe deer were chosen as study species as although they occupy similar habitats they are different in size and represent two different kinds of feeders (Hofmann, 1988). Roe deer are concentrate selectors and fallow are termed intermediate feeders, they possess different morphophysiological characteristics which indicate that they select different diets. Therefore comparisons can be made throughout between the two species and Hofmann's classification verified, but also the body morphophysiology and size model are addressed as means of explaining diet selection.

Although the goal of this study was to answer the main question, several interesting questions emerged at each stage of the analysis these will be addressed and answered within each chapter.

Diet Composition (Chapter 2)

The fact that there were two species of deer of different feeding types suggested that one should have a broader diet in terms of the number of different plant species eaten, or one may eat more of a particular plant species than the other, or more likely a combination of these two occurs. The following questions can be asked:

a) ***Does the number of plant species taken differ over the year and is this different for fallow and roe deer?***

b) ***Does the occurrence of plant species in the diet differ over the year and is this different for fallow and roe deer?***

c) ***Does the quantity of plant species eaten differ over the year and is this different for fallow and roe deer (in terms of total amounts eaten)?***

All of these questions will highlight any differences between the two types of feeder. They also potentially demonstrate selection whether it is the number of species eaten, the occurrence of species in the diet or the amount of each species eaten, they all show how the diet can vary. These quantitative questions are answered using faecal analysis techniques.

Availability of vegetation (Chapter 3)

The availability of vegetation at the fallow and roe study sites was measured as diet selection is very closely linked with availability.

a) ***Is the number of plant species taken over the year by fallow and roe deer related to the number of plant species available?***

b) ***Is the occurrence of plant species in the diet of fallow and roe deer over the year related to the plant species available?***

c) ***Is the quantity of plant species eaten over the year by fallow and roe deer related to the availability of the plant species?***

The answers to these questions would show how much of an effect the availability of vegetation could have on diet selection and consequently how much consideration should be given to it when describing selection processes.

Chemical analysis of vegetation (Chapter 4)

The quality of the diet eaten by fallow and roe deer over the year in terms of the concentrations of nutrients generated its own questions:

a) ***In terms of each plant species eaten, how does the nutrient content change over a year?***

b) ***Of the nutrients analysed, do they all show the same pattern of occurrence throughout the year?***

The answers to these two questions will demonstrate the seasonal availability of nutrients over the year and indicate whether or not there is the opportunity diet selection to take place.

CHAPTER 2 DIET COMPOSITION

ABSTRACT

The diet of fallow and roe deer was investigated using microhistological techniques of faecal material. Two fallow deer sites (Brinken and Denny Lodge) and two roe deer sites (Great Covert and Squabb) were chosen. All four sites were within 15 miles of each other in South Hampshire, UK. The diet composition at each site was recorded for a 12 month period, then subsequently divided into six bimonths (two month blocks). Number of plant species eaten, occurrence and volume of specific plant species were recorded for each bimonth at each site. Seasonal variation in diet composition was evident at each site and was attributable to the life cycles of many plants. The two fallow deer sites showed significant differences in the number and occurrence of plant species eaten, whereas the roe deer sites were more similar to each other. The data clearly suggested that the diets of fallow and roe deer were dependent on their habitat which was reinforced by published accounts of their diet composition. At the fallow deer sites Moss was preferred in winter/early spring together with Holly at Brinken and Pine at Denny Lodge. In late spring/summer these were replaced by Oak at Brinken and Grasses at Denny Lodge. The most preferred forage species by the roe deer were very different at the two sites. Conifers (Western hemlock and Douglas fir) at Great Covert in winter/early spring were replaced with Oak, Grasses and Herbs in summer. At Squabb, Bramble was preferred all year round in addition to Ivy and Pine in winter and Oak in late spring/summer.

2.1 INTRODUCTION

At its most basic level the composition of an animal's diet simply describes what that animal has eaten. If it is measured on more than one occasion, for example over a year, then it is also possible to investigate seasonal variation in what they are eating. The diets of many animals have been described in terms of quantity and seasonality. These basic descriptions can be extremely informative in their own right, however when coupled with more detailed information about what has been eaten a more powerful tool appears, helping to understand situations or problems that animals encounter.

Coupling diet composition with the availability of plant species in an animal's habitat will indicate whether or not they are choosing to eat species that are simply more available. If diet composition is combined with diet quality (nutrient profile) this could suggest whether animals are selecting foods on the basis of nutritional content, toxicity, etc. Diet composition is therefore the cornerstone to understanding more about diet selection in animals. Due to its pivotal role it is imperative that the composition of the diet is accurately recorded, therefore the methodology used is of critical importance. This chapter will record the diet composition of fallow and roe deer and the choice of methodology is discussed in Section 2.1.1.

In this study the primary reason for determining the diet composition of fallow and roe deer was to combine it with availability and nutrient content of the plants eaten to investigate diet selection. The composition of fallow and roe deer diet in the UK, particularly of the former species, have by no means been exhaustively investigated. Therefore this chapter will address this important area that is fundamental to this thesis and extremely important in its own right, before embarking on the question of diet selection. The data collected were very comprehensive as they would ultimately be used to determine diet selection. Consequently the data will be extremely valuable in their own right as there have been very few descriptions of fallow or roe deer diets at the current locations and even fewer in depth studies anywhere in the UK.

Many researchers have looked at the diets of deer using microhistological analysis of faecal (*e.g.* Hosey, 1981; de Jong *et al*, 1995) or rumen samples (*e.g.* Jackson, 1974, 1977; Caldwell *et al*, 1983), some have then considered diet selection from their findings (*e.g.* de Jong *et al*, 1995). The method used for determining diet composition in this study was faecal analysis. An outline of why this method was chosen is given in Section 2.1.1 and a detailed critique is given in Section 2.4.1.1

There have been many studies to determine the diet of deer, but few have looked specifically at the diet of fallow deer in the UK (Jackson, 1974, 1977; Caldwell *et al*, 1983; Kerridge and Bullock, 1991; Putman *et al*, 1993). The most well known and most relevant, is that of Jackson (1974, 1977). This study was carried out in the same locality as the current study, but data were collected by direct observation and rumen samples. The latter method meant that numbers of samples were limited at certain times of the year due to culling restrictions. The Deer Act (1991) stipulates statutory close seasons for the hunting of red, sika, fallow and roe deer in the UK. For male fallow deer this is 1st May - 31st July and for the females, 1st March - 31st October. This explains why the numbers of samples in Jackson's data were low during the months May to July when the author must have relied upon road kills or animals that had died recently from other causes. Putman *et al* (1993) conducted another study in the New Forest looking at sex differences in the diet composition of fallow deer. Caldwell *et al* (1983) looked at the autumn and winter diet of fallow deer in Essex.

In contrast there have been several studies carried out in different parts of the UK describing the diet of roe deer (Hosey, 1974, 1981; Henry, 1975, 1978; Jackson, 1980; Hearney and Jennings, 1983; Johnson, 1984; Sharma, 1994; de Jong *et al*, 1995). Most comparable with this current study are works carried out using faecal analysis to investigate roe deer diet (Hosey, 1974, 1981; Henry, 1975, 1978; Sharma, 1994). Hosey's (1974, 1981) faecal study was based in Chedington Wood in Dorset, which consisted of young softwoods with thick deciduous undergrowth. The deer that Henry (1975, 1978) studied were in Hamsterley Forest in County Durham which comprised coniferous forest planted on moorland. Sharma (1994) studied roe deer in the New Forest in areas of mixed and coniferous woodland. Jackson (1980) also described the diet of roe using rumen analysis with the same limitations as previously mentioned.

Many researchers in Europe, particularly in eastern European countries have looked at the diet of roe deer (*e.g.* Poland: Siuda *et al*, 1969; Kaluzinski, 1982; Czech Republic: Holisova *et al*, 1986; Homolka and Heroldova, 1992; Hungary: Matrai and Kabai, 1989). These studies, although of importance, are not directly comparable with the current study, mainly due to differences in habitat and vegetation availability. This conclusion is reinforced by a recent study by Cornelis *et al* (1999) who assembled all of the research on roe deer diet composition within Europe and explored the influence of season, habitat, research methodology and geographical location. They found that there is relatively little seasonal variation in diet composition and that the changes in what is eaten are influenced more by habitat (*i.e.* the available food items) than by season. They conclude that the influence of habitat is more important than the research method used. As a consequence in this study the composition of roe deer diet at Great Covert and Squabb will be compared with the published reports of Hosey (1974, 1981), Henry (1975, 1978), Sharma (1994) (all faecal analysis) and Jackson (1980; rumen analysis).

2.1.1 Choice of method

The most simple technique to determine diet composition is to actually observe what the animal eats (*e.g.* Jackson, 1974). This technique relies upon the observer being close enough to view the animal and the vegetation. This method is probably most useful if only the taxonomic levels of genus or family of the plants are required. Identifying different grass species from a distance, for example, is very difficult, although some authors have claimed to have done this (*e.g.* Jackson, 1974). Wallmo *et al* (1973) state that this method is not always accurate and suggest that the observer must be within 23m of the deer to identify 80% of the grazed plant species correctly.

Another technique used to determine herbivore diets is to fistulate animals (*e.g.* Talbot, 1962; Hansen *et al*, 1973; Holechek *et al*, 1982). This is an invasive method requiring an expensive surgical operation where a tube is inserted directly into the oesophagus or the rumen and ingested or partially digested material can be taken out at any time. This method relies upon having captive animals and is therefore of limited use when investigating the natural diet of herbivores. It does however produce precise and accurate results, and so is often used to test the reliability of other methods. It is also a useful method for looking at variables related to digestion.

A further method, briefly mentioned earlier, is rumen content analysis (*e.g.* Siuda *et al*, 1969; Short, 1971; Jackson, 1974, 1977; Henry, 1978; Jackson, 1980; Kaluzinski, 1982; Caldwell *et al*, 1983) which requires the animal to have died whereupon the rumen and its contents are removed for detailed analysis. Most authors that have used this method have either killed animals or have made use of seasonal culling, hunting or road kills. While this method allows determination of the animal's natural diet it restricts the description to the time the animal was killed. In some cases, rumen samples can be obtained throughout a season or possibly a year to obtain a fuller profile of the diet, but even in these cases there are often insufficient numbers of animals to give the results credibility. This method is therefore also of limited use, it provides glimpses into the diet but could not be used for rare or endangered animals.

A common method used for determining diet composition is microhistological analysis of faecal material; the identification of plant fragments (at cellular level) that have passed through the digestive system. The reliability of this technique has been criticised widely by many authors; does it give an accurate representation of the entire diet? Discussions are ongoing, but it does have many advantages which have been listed by Anthony and Smith (1974) and Holisova *et al* (1986) and include: allowing unlimited, continuous sampling; samples are easy to collect, preserve and store; there is no disturbance to the population and most importantly the animals do not have to be killed. The main disadvantages (in addition to the ongoing discussion regarding its reliability as a method) are that it is very time consuming and labourious, and usually no information is obtained about the sex, age or physical condition of the animal, unless they are observed defecating (Anthony and Smith, 1974; Holisova *et al*, 1986).

A summary of benefits and drawbacks of each method discussed can be found in Cornelis *et al* (1999). Smith and Shandruk (1979) carried out a comparison of the different methods using pronghorns (*Antilocapra americana*) and mule deer. They found that, for diet composition, the methods in closest agreement were faecal analysis and actual observation of the animals, suggesting that faecal analysis is an accurate, practical technique. However there continues to be doubt about the value and applicability of faecal analysis and many authors have assessed the differences between faecal and rumen analysis (*e.g.* Martin, 1955; Stewart, 1967; Zyznar and Urness, 1969; Todd and Hansen, 1973; Anthony and Smith, 1974; Dearden *et al*, 1975; Takatsuki, 1978; Smith and Shadruk, 1979; Barker, 1986; Homolka and Heroldova, 1992; Padmalal and Takatsuki, 1993). Anthony and Smith (1974) found that the frequency of plant fragments was higher in faecal analysis than ruminal, and different species were found using the two methods. Thus, many researchers claim that faecal analysis can only produce qualitative information and not quantitative (*e.g.* Martin, 1955; Smith and Shadruk, 1979). In contrast, a study by Homolka and Heroldova (1992) found the mean number of food items in faecal and stomach samples were not significantly different, all the main food components, except fungi, were identified in both sets of samples. These authors recommend both methods for dietary studies on free ranging ungulates. Padmalal and Takatsuki (1993) also believe it to be a promising method for quantitatively estimating the diet composition of deer.

From the literature there does not appear to be a clear consensus of opinion as to whether faecal analysis is a useful tool or not, it appears to be reliant on the practicalities and suitability of the experimental situation. For many researchers the advantages outweigh the disadvantages and this was also the case for this present study. This technique was chosen because the diet composition of both fallow and roe deer was to be assessed on a monthly basis, at the same sites throughout. Rumen samples were not an option as 10 samples (equating to 10 deer, see Section 2.2.2) would be needed each month to give a unbiased profile of the diet, so rumen analysis would mean the unnecessary and untimely death of many animals. Furthermore the closed hunting seasons must be adhered to when considering rumen analysis. Stewart (1967) and Storr (1961) emphasise that faecal analysis allows periodical resampling of a population to investigate precisely the fluctuating relationships between diet and availability. This was precisely the relationship investigated in this current study and so another justification for using faecal analysis techniques.

2.2 METHODOLOGY

This section describes the study sites (Section 2.2.1) and outlines the collection (Section 2.2.2), preparation (Section 2.2.3) and analysis of sample material (Section 2.2.4). Finally Section 2.2.5 describes how the data were analysed and which statistical tests were employed.

2.2.1 Study sites

Samples were collected from two study sites for each of the two species of deer. The sites were specifically selected as they were known to predominantly contain only the subject species of deer (R.J.Putman pers.comm. and personal observations), thereby avoiding misidentification of faecal material. The sites chosen represented the range of suitable habitat for each species, however limiting the number of sites to two per deer species does have disadvantages. Instead of focussing on differences between the two species of deer as a main effect, the data analyses could only address differences between sites and bimonths using the model: *response = site + bimonth + site*bimonth.* Two possible alternatives were considered but both also had disadvantages:-

a) selecting at least six sites per species and using the model: *response = deer + site(deer) + bimonth + bimonth*deer + site(deer)*bimonth.* However, in order to limit variation in plant availability, soil type etc. the twelve study sites would have had to be in the same geographical area which would have been very difficult to achieve. Also, due to time and financial constraints the overall study of nutrient selection would not have been as detailed as required.

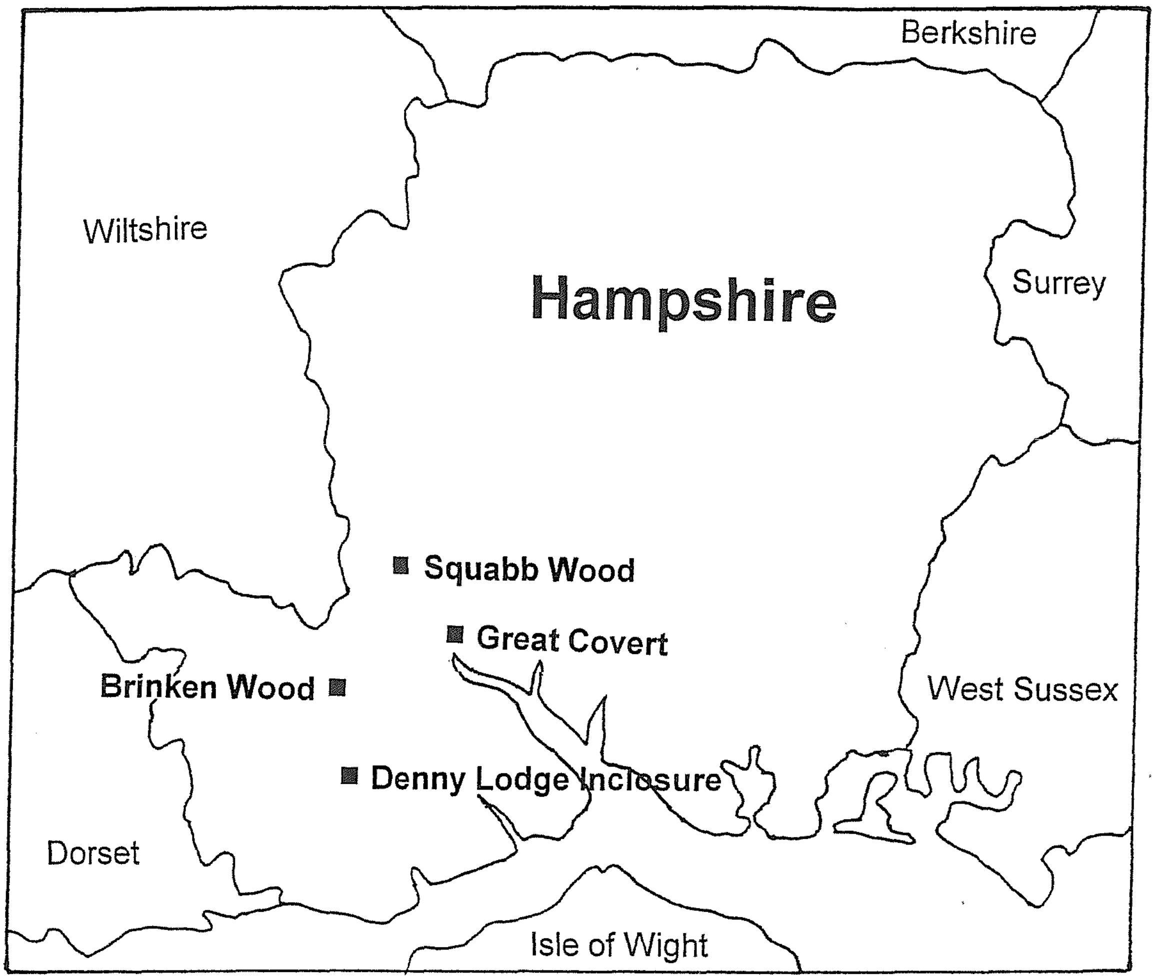
b) selecting sites that contained both fallow and roe deer, however this may have created problems in identifying faecal samples and would also bring in effects of competition.

The four sites chosen were located in Hampshire in southern England (Figure 2.1).

2.2.1.1 Fallow deer sites

The fallow deer sites were both in the New Forest, Hampshire. This is an area of woodland, heathland, grassland and marsh covering 37,907ha (Tubbs, 1988). There are four major species of deer in the New Forest; fallow deer, roe deer, red deer and Japanese sika deer. More recently, and

Figure 2.1 Map of Hampshire showing the positions of the four study sites.



after this study was started, there have been sightings of muntjac (*Muntiacus reevsi*).

The first fallow site was Brinken Wood (OS grid reference SU274064; Figure 2.2), this is an ancient woodland containing Beech (*Fagus sylvatica*), Oak (*Quercus robur*) and Holly (*Ilex aquifolium*). There are no permanent thoroughfares but there are grass covered pathways used by walkers. This woodland is bordered on one side by open heathland. The core area used for the study covered the northern part of the wood and most of the heathland, approximately 35ha.

The second fallow deer site was Denny Lodge Inclosure (SU344041; Figure 2.3), an area consisting largely of coniferous woodland of Pine[1] (*Pinus* spp.), Douglas fir (*Pseudotsuga menziesii*) and Western hemlock (*Tsuga heterophylla*). There are also areas of Beech and Oak woodland. The wood is transected by grassy rides and Forestry Commission purpose laid tracks. The surrounding areas are made up of marshy heathland and coniferous woodland. The study area over which collections and observations were made covered the central part of the woodland and was approximately 55ha in size.

2.2.1.2 Roe deer sites

Due to the low numbers of roe deer in the New Forest (Sharma, 1994), the study sites for this species were privately owned woodlands. One site was Great Covert, North Baddesley, Hampshire (SU413199; Figure 2.4). This is predominantly a coniferous woodland, consisting of Western hemlock and Douglas fir, but there is also a narrow band of deciduous trees, mainly Silver birch[2] (*Betula pendula*) running alongside the main road (Hogstrough Wood). There are several grassy rides through the site. The surrounding area is mainly farmland; fields of grass and sprouts/cabbages. The main study area consisted of approximately 75ha taking in the woodland and the rides but excluding the neighbouring fields.

The second roe site was Squabb Wood, part of the Broadlands Estate, Romsey, Hampshire (SU335220; Figure 2.5). This is a mixed woodland comprising areas of deciduous trees, mainly Birch but also Beech and Oak, and areas of coniferous trees predominantly Douglas fir and Western hemlock. This site has grassy tracks crossing it and opens out on to a large arable area, which at the

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[1] predominantly Scot's Pine, referred to as Pine throughout this thesis

[2] Silver birch will be referred to as Birch throughout this thesis

Figure 2.2 Brinken Wood study site (SU274064) in the New Forest, Hampshire (edged in red). Source: Ordnance Survey Outdoor Leisure 22.

Scale 5.6cm:1km

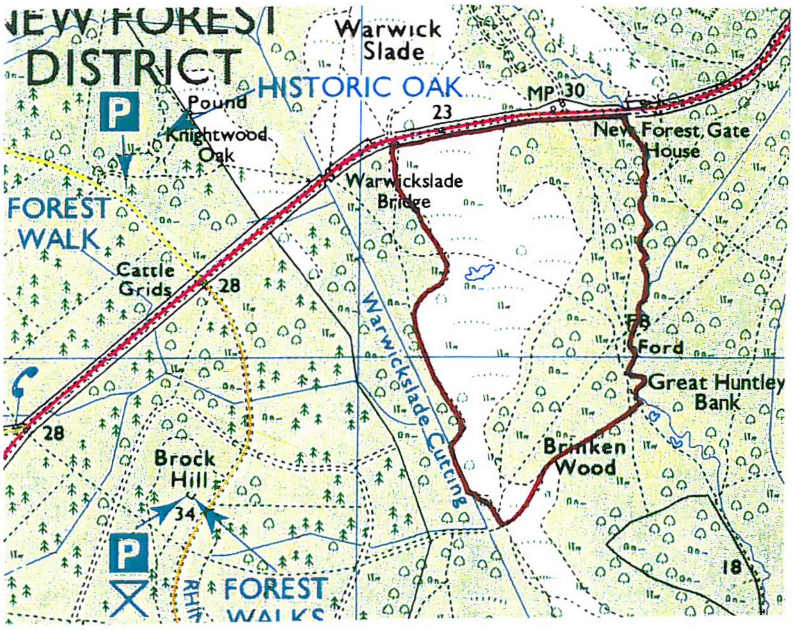


Figure 2.3 Denny Lodge Inclosure study site (SU344041) in the New Forest, Hampshire (edged in red). Source: Ordnance Survey Outdoor Leisure 22.

Scale 5.6cm:1km

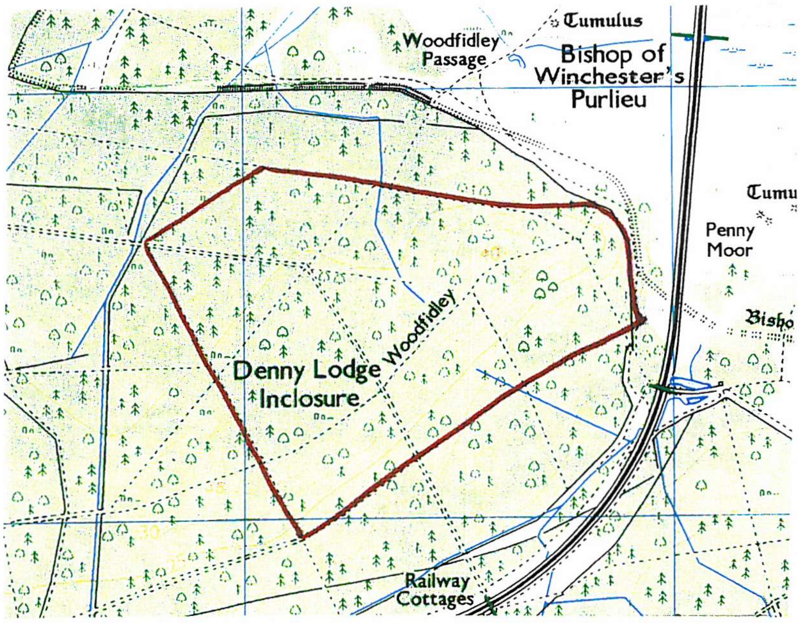


Figure 2.4 Great Covert study site (SU413199) North Baddesley, Hampshire (edged in red).

Source: Ordnance Survey Pathfinder 1264

Scale 10.7cm:1km

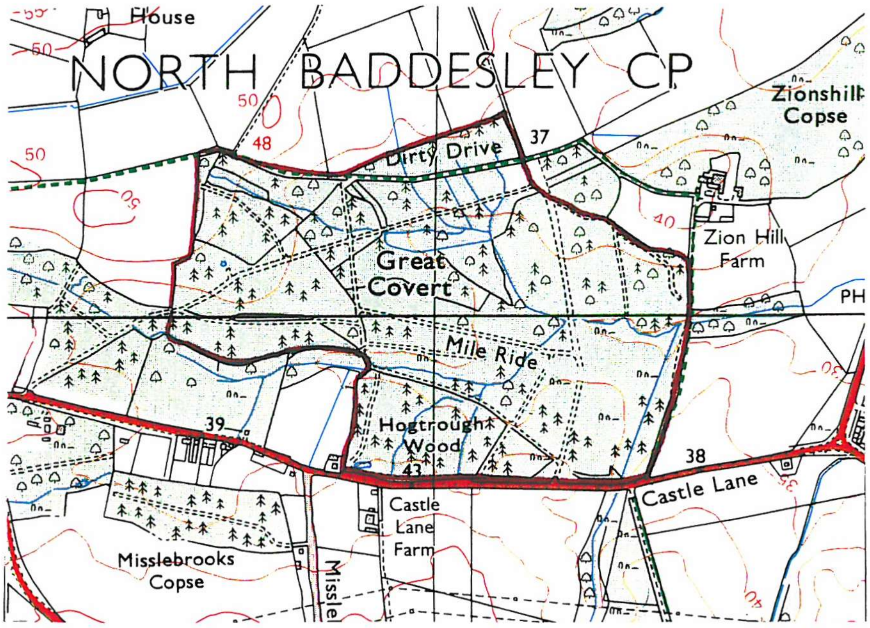
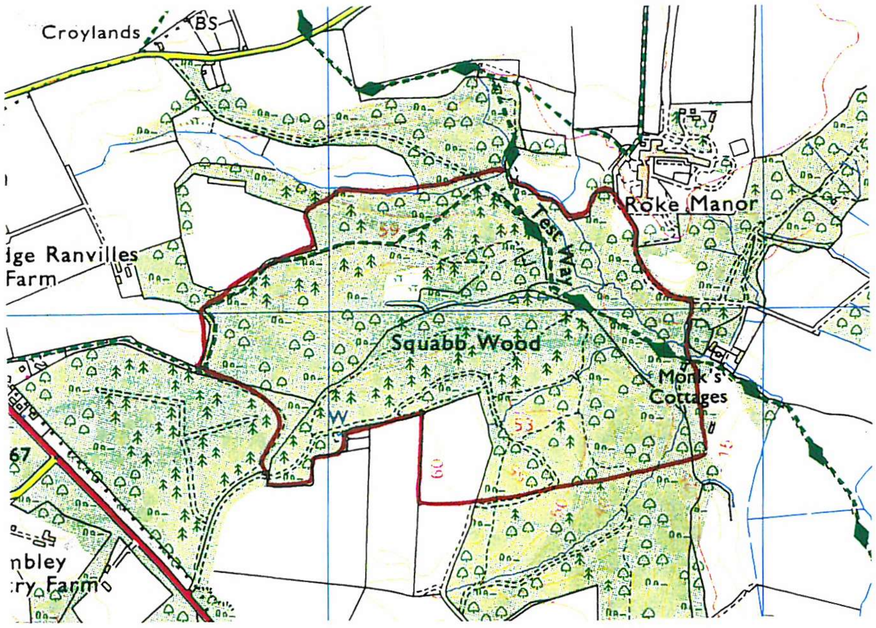


Figure 2.5 Squabb Wood study site (SU335220) Romsey, Hampshire (edged in red).

Source: Ordnance Survey Explorer 131.

Scale 5.6cm:1km



time of the present study, was planted with Oil seed rape (*Brassica napus*) and Wheat (*Triticum aestivum*). The woodland was the main study area and covered approximately 70ha.

2.2.2 Collection of faecal samples

The four sites were visited once a month for one year. For the fallow sites the study year was January 1990 to December 1990, for the roe it was September 1990 to August 1991. The difference in start dates for the two species of deer was due to the difficulty in finding suitable roe sites and obtaining permission from the appropriate governing bodies as the sites were privately owned. Visits were made every calender month and were timed such that there was at least three weeks between each visit.

Every month the study area of each site was walked by a single experimenter, initially this was done in a random manner but after 2-3 months areas frequented by the deer became apparent. Samples of fresh faecal material were collected. At certain times of the year, in mild conditions roe faecal pellets are reported to remain on the ground for 3-5 months (Mitchell *et al*, 1985). Therefore only the freshest samples could be guaranteed to come from the particular month being studied. Fresh samples had to be shiny (mucus) and black, any that were greenish, dull and swollen, as a result of rain, or dry and shrunk were disregarded. Samples of fresh faecal material were often insured by disturbing individuals or groups of deer and then surveying the area from which they had fled. Ten pellets were collected each month from ten different areas within each study site (approximately 10m or more apart). This method attempted to represent ten individual deer (see Stewart and Stewart, 1970; Hosey, 1974). Although, different faecal pellet sizes have been reported for male and female fallow deer (Chapman and Putman, 1991), the differences are very small and therefore not used in this study. In addition, age was also undetermined, on occasion very small pellets were observed and assumed to indicate young animals, these were not collected in an attempt to remove any effect of age. Each faecal pellet sample was indirectly handled and was collected into a plastic bag, in which the pellets remained for a maximum of a three hours before being transferred into separate labelled paper bags in the laboratory.

The faecal samples were air dried in the paper bags at room temperature. It was important not to place the samples straight into the paper bags if they were wet, *i.e.* from rain or dew, as this could encourage microbial decomposition. In these cases the samples were dried at room temperature for up to four hours depending on how wet they were, by placing them in a tray.

The bags were regularly checked for signs of microbial contamination and breakdown, on finding signs of such activity the samples were immediately discarded, but the actual occurrence was very low (only 12 samples out of 464 were lost in this way). In the summer months the faecal samples were checked within hours of placing them in the paper bags as dung beetles (*e.g. Aphodius* spp.) often laid their eggs in the faecal pellets and the larvae soon hatched and started to breakdown the samples. Generally these insects were removed before they had done any damage but on eight occasions they had broken down the material significantly, these samples were discarded. Once the faecal samples were dried the bags were gathered into monthly groups and kept in a cool, dry store, awaiting analysis.

2.2.3 Preparation of faecal samples

One of the first problems encountered with faecal analysis is the **discernability** of the plant fragments, the lack of clarity when first viewed under the microscope. The sample is usually too dark to see any plant fragments and this has led to the formulation of a number of preparation methods.

The aim of the preparation methods is to remove the mucus surrounding the faecal material and also some of the colour acquired through the digestion process whilst leaving the cellulose cell walls intact. Early methods involved boiling the faecal material in an acid or acid mix (nitric acid with or without chromic acid) followed by washing (Storr, 1961; Stewart, 1970; Stewart and Stewart, 1970).

Other researchers soak the material overnight in a dilute sodium hydroxide (NaOH) solution before stirring and washing (Zyznar and Urness, 1969). Sodium hydroxide solution has been tested along with other chemicals and was found by Holechek (1982) to be the best preparation agent when used with Hertwigs clearing solution (see Holechek, 1982). Vavra and Holechek (1980) also found that NaOH in conjunction with other facilitating methods (*e.g.* grinding) improved the accuracy of diet estimation, particularly for grasses. The NaOH solution on its own gave reduced sensitivity with grasses but analysis of shrubs and forbs was improved (Vavra and Holechek, 1980). Acids were not used in the present study, using NaOH was found to be a quick productive method and each faecal sample was prepared using the following method.

Four to five faecal pellets were ground using a pestle and mortar (Storr, 1961) with 4-5 drops of 2% NaOH solution as a lubricant. This was then transferred to a beaker and approximately 50ml of the

NaOH solution was added. The faecal material was left to soak in the solution for 18-24 hours, allowing the solid matter to settle and most of the colour to be removed. The supernatant was poured away and discarded and the solid matter was washed with distilled water several times. Between each washing the solution was spun in a centrifuge for 20-30 seconds and the supernatant discarded. This was done to remove dust that can seriously obscure the plant fragments (Putman, 1984). Washing continued until the supernatant was clear which was usually after 2-3 washes. After the final wash the supernatant was poured away leaving semi-solid material.

2.2.4 Analysis of faecal samples

2.2.4.1 Method used

A Pasteur pipette was used to place a sample of the semi-solid material onto a petri dish, to which a few drops of water were added, sufficient to just cover the bottom of the dish and suspend all the plant fragments. The petri dish had a grid (5mm x 5mm) scored on to the underside. The sample was placed under a binocular microscope (Kyowa Optical model SDZ-PL) fitted with a x2 converter giving x90 magnification. The effect of magnification used for identification purposes has been investigated by Holechek and Valdez (1985), who found no difference between x100 and x200 in terms of accuracy. The magnification in the present study was used as it was the highest possible on a microscope that could take a petri dish and according to Holechek and Valdez (1985) it was acceptable.

The method used for recording the fragments was adapted from Storr (1961) and Stewart (1967). The scored lines were traversed and any plant fragments touching a line were identified. Fragments of vegetation were identified by their characteristic cell walls; their shape and how they fitted together, using reference material (supplied by University of Southampton) that had been collected from similar sites (in the New Forest), digested *in vitro* and photographed showing the cell walls. Fragments were identified to species level where possible (Appendix 1). The first 100 fragments touching the lines were identified and recorded. If any fragments could not be identified to species level they were taken down to genus, family or order level or in some cases Monocotyledon or Dicotyledon (Appendix 2). Potentially identifiable plant fragments (*i.e.* usually one cell thick and large enough to see a number of cells) that could not be identified to any level were categorised as unknown.

In the first instance three sets of 100 fragments were identified for each faecal sample, these were compared to check the reliability of identification. Once consistency had been achieved 300 fragments were identified from each sample, however for ease they were still done in three blocks of 100. This was done for each month providing a monthly total of 3000 fragments[3] (10 samples were usually collected each month). Data were recorded as the numbers of fragments for each plant species.

2.2.5 Data preparation and analysis

2.2.5.1 Bimonth groupings

The data collected from the faecal analysis were very extensive. Therefore it was necessary to condense them before statistical analysis could be carried out. The simplest way to do this was to combine data from two months producing a bimonth grouping thereby reducing the data by half. This could be achieved in two different ways:-

A	JanFeb	MarApr	MayJun	JulyAug	SepOct	NovDec
B	DecJan	FebMar	AprMay	JunJul	AugSep	OctNov

There was no obvious benefit to or preference for either set of bimonth groupings. It was therefore necessary to examine both options to see whether or not there was any difference between them.

Each month, at each site, generally consisted of ten samples, and from these, 3000 fragments were identified. Thus, each bimonth should have consisted of 6000 plant fragments. However, due to the problem of misidentification of deer hair as a plant fragment (see footnote) the total number of true plant fragments in each month varied.

In order to explore the problem of bimonth grouping, plant species were chosen that had a total of at least 300 fragments (approximately 1%) across the year. The numbers of fragments of each plant species was thought to be the most variable measure across the bimonths and the sites so was used to test the two sets of bimonth groupings. The number of fragments identified of each species were

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[3] unfortunately deer hair was identified throughout as a plant fragment and as this is not plant material its occurrence had to be removed from each of the monthly samples, therefore the total number of fragments for each month was different depending on how many hair fragments had been present.

calculated for each of the 12 different bimonth combinations (A and B) this created two separate sets of data for each plant species for each site.

The two combinations (A and B) for each plant species were tested at each site using a Kolmogorov-Smirnov test (see Section 2.2.5.6) and the differences and probability levels compared. All the tests produced maximum differences between the observed and expected values for each plant species in each bimonth combination, creating two new data sets. As none of the Kolmogorov-Smirnov tests were significant a parametric Paired t-test (see Section 2.2.5.6) was used on the two sets of data to see if there was any significant difference between the two possible combinations of bimonths (A and B) at each site (Brinken N=17; Denny Lodge N=9; Great Covert N=11; Squabb N=8). The null hypothesis being tested for each site was:-

H_0 = no significant difference exists between the two sets of bimonth combinations

The Paired t-test gave non-significant differences for each site (Brinken $t_{16} = -1.809$, NS; Denny Lodge $t_8 = -0.241$, NS; Great Covert $t_{10} = -1.382$, NS; Squabb $t_7 = 2.04$, NS) and therefore the null hypothesis was accepted. Thus, using one set in favour of the other would have no consequences in any further analyses.

For all the faecal data analysis the monthly samples were arbitrarily combined to make bimonthly groups as follows: January and February (JanFeb); March and April (MarApr); May and June (MayJun); July and August (JulAug); September and October (SepOct) and November and December (NovDec).

2.2.5.2 Data analyses and considerations

The faecal data for each site contained many different species of plants (Appendix 1). The groups of plants used for all four sites ranged from genus level through to very broad categories such as Dicotyledon. Appendix 2 shows the names of the groups and of what they comprised.

The data collected from faecal analysis were explored in three separate ways:-

1. The **number** of plant species found in the faeces at each of the four sites and over the year (Section 2.3.1). A description of this analysis and the conditions imposed are found in Section

2.2.5.3.

2. The **occurrence** of different species (groups) of vegetation in the faeces at each of the four sites and over the year, or the number of deer eating the different species of vegetation at each site over the year (Section 2.3.2). The criteria for including data in this analysis together with a description of the measure are in Section 2.2.5.4.

3. The **proportion or volume** of each plant species (group) in the faeces at each of the four sites and over the year (Section 2.3.3). A description of this measure and how it was calculated are in Section 2.2.5.5.

2.2.5.3 Number of plant species found in the faeces

This measurement was of interest for two reasons. Firstly for measuring diversity at each site as this will directly relate to the numbers of plant species that were available (Section 3.3.2). Secondly, it will investigate if the two different kinds of feeders (fallow deer: predominantly grazers; roe deer: concentrate selectors, Section 1.2.2) took in different numbers of plant species or if there was no difference.

The analysis of the number of plant species found in the faeces did not include the vegetation groups Dicotyledon, Monocotyledon, Grass, Coarse Grass and Deciduous tree. In this case the actual identity of the plant species was not of interest and it was very likely that each of the groups listed above would have been represented by at least one of the actual species it contains (Appendix 2). By doing this a possible overcount in number of species was avoided. The group Herb was retained for this analysis as this group was very difficult to identify down to species level using the faecal analysis technique, and consequently to exclude this group would have misrepresented the dietary intake of the deer.

The total number of different plant species was calculated from each bimonth group which consisted of twenty[4] samples (ten samples from each month). All data were used for further analysis with the following conditions:-

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[4] number of samples in each bimonth ranged from 12 to 20, although 20 was the mode.

a) **if only one deer ate a particular species it had to have more than one fragment in its faecal sample**

or

b) **if only one fragment was found in one sample it had to have been eaten by at least one other deer (*i.e.* be present in one other sample)**

The presence of a plant species outside these conditions was considered to be a chance consumption, *i.e.* not deliberately eaten by the deer. Exploration of the data indicated that there was no difference in the numbers of 'chance' plant species eaten by the two species of deer or at each of the four sites (deer: Mann-Whitney $U_1 = 256.5$, NS; site: Kruskal Wallis $\chi^2_3 = 1.834$, NS). These findings made the omission of these data at each site acceptable.

2.2.5.4 Occurrence of plant species eaten

Occurrence measures presence and absence of particular plant species in the diet, it gives an indication of the importance of each plant species in the diet, *e.g.* if Holly occurred in the diet of 75% of the fallow deer samples (15 deer) it would be considered important. Even though there were no data on how much was eaten the observation that many deer ate it suggests significance. Measures of occurrence can also be related to the availability (Section 3.3.3).

The frequency of particular plant species occurring was measured by recording the number of samples in which each species of vegetation was found. The groups of plants (Appendix 2) were included in this set of data, therefore in this case when the occurrence frequency is referred to in terms of species it also applies to the groups of vegetation. Each bimonth consisted of usually twenty samples (range 14-20) thereby giving a maximum occurrence of 20 for each plant species. As each sample was assumed to be one individual deer this could also be a measure of how many deer ate a particular plant species. However, this term must be used loosely as the samples were taken from two separate months and it is not possible to eliminate the possibility that some, if not all, of the faecal samples of both months (within a bimonth) came from the same deer.

The criteria for data inclusion were that a particular species of vegetation had to **occur in at least two of the three replicates taken from each faecal sample ('deer')**. One fragment in three replicates could easily have been an incorrect identification and therefore unreliable to include in further analysis. However, two fragments in three replicates suggested that the identification was correct and that the particular plant species in question was present in small quantities in the faecal sample. In addition, the plant species that were included for analysis were required to have occurred in **two or more samples (~ eaten by more than two deer) *and* in two or more bimonths within the year** (Appendix 3).

These criteria eliminated plant species that had only been eaten in one bimonth by one deer, the occurrence of these plant species could not be reliably compared over the year and across the sites.

2.2.5.5 Volume (proportion) of plant species eaten

Volume measured the proportion of the whole diet that was composed of a particular species or group of plants and was calculated as follows:

$$\text{Volume} = \frac{\text{number of fragments of Species X in Bimonth Y}}{\text{total number of fragments in Bimonth Y}} \times 100$$

This gave the proportion of Species X eaten by one deer in Bimonth Y. The mean bimonth proportion of each species or group of plants was calculated for all the samples collected (range 14-20) taken each bimonth. This was determined for each site (Appendix 4: Tables A4.1 - A4.4). The data have been organised into three sections: plants contributing to ≥5% of the diet; ≥2% of the diet (but <5%) and ≥1% (but <2%) in any one bimonth across the year. Any plants making up <1% of the diet were disregarded and not used in any statistical analysis.

The volume of individual species within the diet of these deer was obtained primarily to investigate diet selection (Chapter 5) based upon the nutrient content of each species (Chapter 4). However the data on the amounts eaten also indicated a plant species' importance in the diet, although to more accurately define importance the volume and occurrence of a particular plant species should be considered together. A species could be eaten in high amounts therefore appearing important but the occurrence data could indicate it was only eaten by one deer and so may not be as important as first thought. Both measures were considered for all the data and were found to be directly related, thus high volume signified importance in the diet as did a high occurrence. Therefore comparisons at an individual species level could also be made between the fallow sites (Brinken vs Denny Lodge) and between the roe sites (Great Covert vs Squabb). For these comparisons it was necessary to only use plant species common to both samples being compared.

The volume data can also be used to make comparisons with published data on the diets of deer. This would allow the current study data to be put into context and the reliability of the faecal analysis technique to be judged by assessing if fallow and roe deer diets contained plant species common to other studies.

2.2.5.6 Statistical analysis of the data

The statistical analysis used both non-parametric and parametric tests. In order to use parametric tests the data must be approximately normally distributed (Siegel and Castellan, 1988). The distribution of the variables in each case was examined to make sure it was normal by displaying the data as a histogram. If it was not normal then the variables were either transformed (see individual results sections for details) to normalize the data or non-parametric tests were used. Non-parametric statistics require little or no knowledge of the distribution of the data.

All hypotheses were tested using a critical probability (P) value of 0.05, unless otherwise stated. The following descriptions of each analysis is based on SPSS Base 7.0 for Windows, User's Guide (1996), Fowler *et al* (1998) and Dytham (1999).

Analysis of Variance (ANOVA): Parametric test used to examine the effect of independent variables (in the case of two-way ANOVA two independent variables) upon a single dependent variable. The independent variables divide the population into groups. ANOVA tests null hypotheses about the main effects of independent variables by comparing the means. It can also be used to investigate interactions between the independent variables.

After determining that differences do exist among the means, **post hoc range tests** are used to determine which means differ. Range tests identify homogeneous subsets of means that are not different from each other. **Duncan's multiple range test** was used throughout because of all the possible post hoc tests this is the most conservative (Steel and Torrie, 1980).

Paired t-test: Parametric statistical test used on data that are paired, *e.g.* when an individual or sampling station is tested twice. It is performed on data that are continuous and approximately normally distributed. The variances of the two sets of data must be homogeneous which can be tested using the Levene test. This t-test looks at the difference between each matched pair and then calculates a mean difference which can then be tested. $P<0.05$ indicates that the two groups of data are significantly different from one another.

One Sample Kolmogorov-Smirnov test: Non-parametric test compares experimental data with expected or theoretical distributions, it calculates the differences between the observed (experimental) and the expected cumulative distributions. It also tests for normality, if the test produces a probability score of less than 0.05, then the distribution of the data is significantly different from normal and requires transformation or non parametric statistical analysis.

Mann-Whitney U test: Non-parametric rank test, the data are converted to ranks before the test is performed, so can be measured on ordinal or interval scales. It determines whether two independent samples are from the same population by comparing the medians. The U statistic is calculated by the number of times a value in the first group precedes a value in the second group, when values are sorted in ascending order. P<0.05 indicates that the samples are from different populations.

Kruskal Wallis test: Non-parametric rank test like the Mann-Whitney test and data can be interval or scale measurements, frequencies, derived variables (*e.g.* proportions) or ordinal ranks. However, whereas the Mann Whitney test only compares two samples this test compares three or more independent samples, with a null hypothesis that all the samples are taken from populations with the same median. In this way it is considered to be the non-parametric equivalent of the one-way ANOVA. P<0.05 indicates that the samples are from different populations.

Log linear analysis: Used to analyse contingency tables with three or more dimensions. The dependent variable is the number of cases (frequency) in a cell of the crosstabulation, and the explanatory variables are factors and covariates. The procedure can be used to test null hypotheses that there are no interactions between variables upon the observed frequencies, or hypotheses of partial and conditional independence.

2.3 RESULTS

2.3.1 Number of plant species eaten

The highest number of different species of plants eaten was found at Brinken, with fallow deer eating an overall mean of 16.29 plant species or groups (N = 117 samples). Denny Lodge, the second fallow site, also showed high numbers of plant species eaten, with an overall mean of 12.94 (N = 120). Great Covert and Squabb showed similar overall means and ranges of the numbers of species eaten by roe deer (Great Covert: mean = 10.56, N = 110; Squabb: mean = 10.9, N = 105).

Figures 2.6a and b show the mean number of species eaten at all four sites over the six bimonths. The overall means at each site and for each bimonth suggested that there may be a difference between sites and also between bimonths, this was tested statistically.

The distribution of the number of plant species eaten was examined for each site and was found not to be normally distributed. The data for all four sites was therefore normalised using a square root transformation and then analysed using Analysis of Variance (ANOVA).

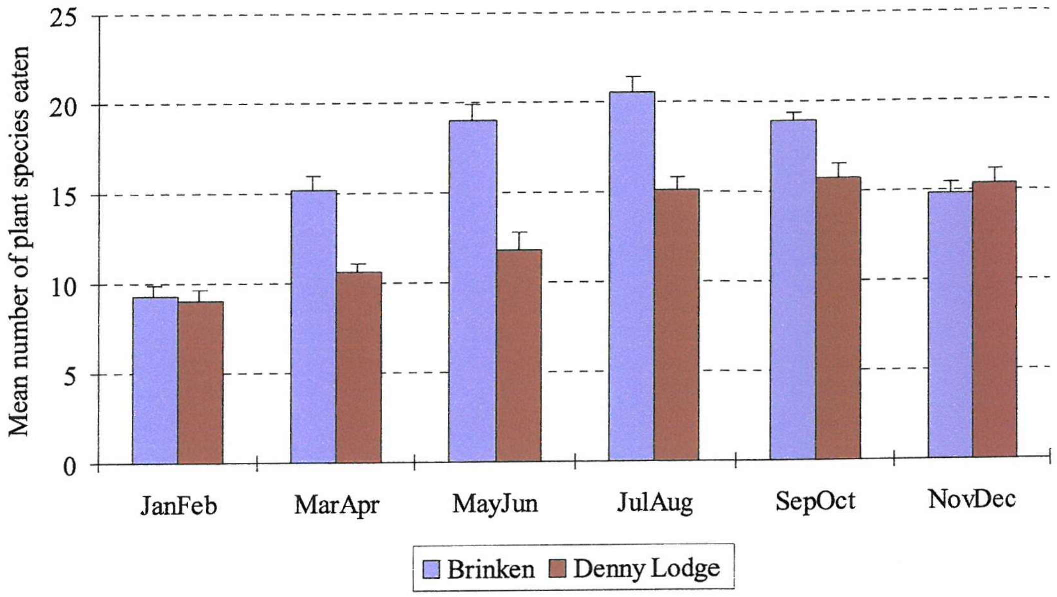
In this case a two-way ANOVA was used to test for significant differences between the sites and also between the bimonths. The null hypotheses being tested were:-

1. H_0 = no significant difference exists between the four sites with respect to the number of plant species eaten by the deer.
2. H_0 = no significant difference exists in the number of plant species eaten by the deer over the year (six bimonths).
3. H_0 = no significant interaction exists between the different sites and the bimonths.

The ANOVA showed that there was a significant interaction between site and bimonth ($F_{15,428}$ = 4.153, $P<0.001$), thus the main effects of site ($F_{3,428}$ = 62.731, $P<0.001$) and bimonth ($F_{5,428}$ = 43.225, $P<0.001$) were significant. All three null hypotheses were rejected. The multiple range test (Duncan) performed on the transformed means for site showed that the roe sites were not significantly different from one another, their mean scores were very similar. Moreover, the fallow sites were not only significantly different from the roe sites, but also from each other (Table 2.1)

Figures 2.6a-b Mean number of plant species (± standard error) eaten over the year at each of the four sites.

a) Fallow deer - Brinken and Denny Lodge



b) Roe deer - Great Covert and Squabb

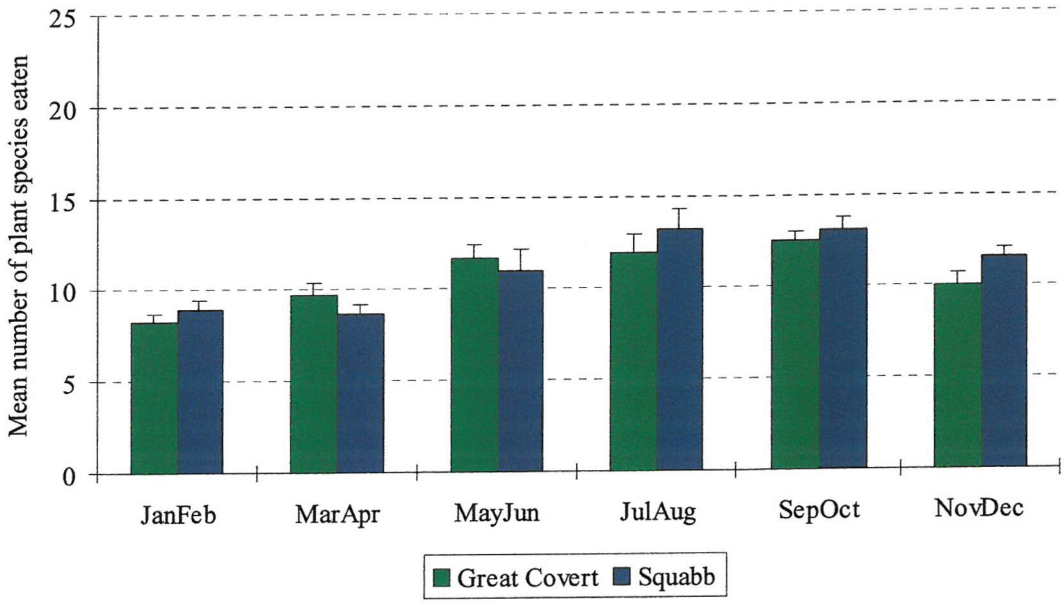


Table 2.1 Multiple range test (Duncan) for the effect of **site** displaying true mean values for number of species eaten (means followed by the same letter are not significantly different from each other).

Site	Mean value
Brinken	15.87 a
Denny Lodge	12.60 b
Great Covert	10.32 c
Squabb	10.57 c

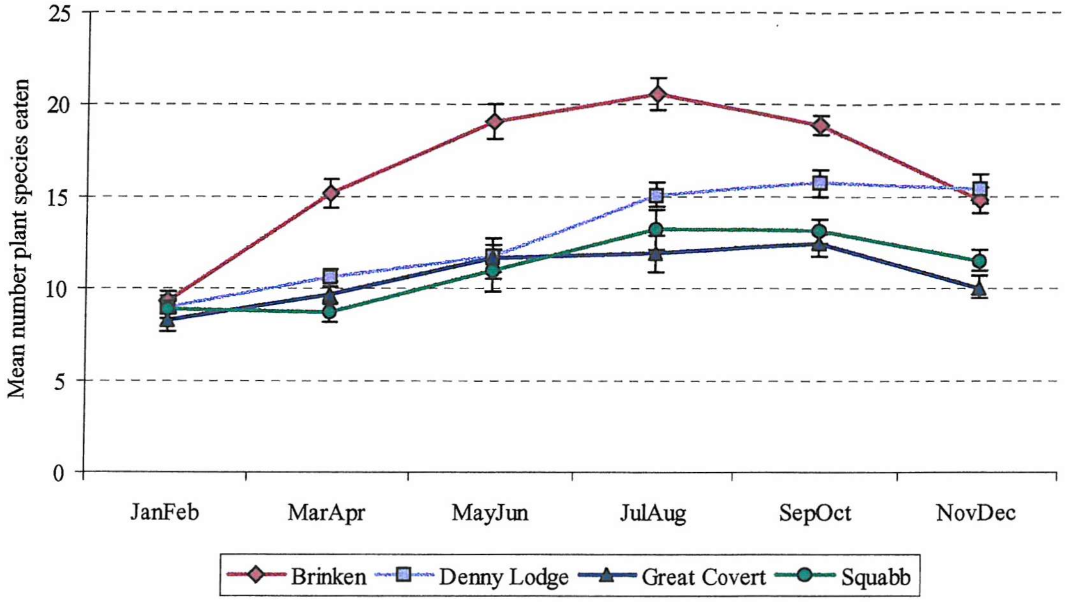
A multiple range test (Duncan) on the transformed means for bimonth (Table 2.2) revealed that JanFeb was significantly different from the other bimonths, it was time of year when the deer took the least number of plant species. In contrast, the summer months of JulAug and SepOct were significantly different to the rest of the year and were when they took the most.

Table 2.2 Multiple range test (Duncan) for the effect of **bimonth** displaying true mean values for number of species eaten (means followed by the same letter are not significantly different from each other).

Bimonth	Mean value
JulAug	15.02 a
SepOct	15.02 a
MayJun	12.86 b
NovDec	12.72 b
MarApr	10.75 c
JanFeb	8.70 d

Figure 2.7 shows the significant difference between the sites over the year with the two fallow sites (Brinken and Denny Lodge) showing the greatest variation over the year.

Figure 2.7 Distribution of mean number of plant species (± standard error) eaten over the six bimonths at the four different sites, demonstrating the significant bimonth by site interaction



2.3.2 Occurrence of plant species eaten

The occurrence of plant species in the diet of fallow and roe deer at each site (Appendix 3) was statistically analysed using Log linear Analysis (Statgraphics 7.0: STSC. Inc, 1988).

Log linear analysis considered two separate sets of comparisons made for each plant species:

1. between the fallow deer sites, *i.e.* Brinken and Denny Lodge
   H_0 = there was no significant difference in the occurrence of each plant species between the two fallow sites.
2. between the roe deer sites, *i.e.* Great Covert and Squabb
   H_0 = there was no significant difference in the occurrence of each plant species between the two roe sites.

The occurrence data were displayed as presence/absence values for each qualifying plant species. For each of the above sets of comparisons the following variables were tested:- site, bimonth and presence/absence (of particular species or groups of vegetation). An example of how the data were presented for analysis is shown in Tables 2.3a-d.

Tables 2.3a-d Examples of contingency tables used for Log linear analysis. These data represent the numbers of times Holly occurred in the diet of fallow deer at Brinken and Denny Lodge in each bimonth period.

a. Brinken

	presence	**absence**
JanFeb	20	0
MarApr	20	0
MayJun	14	5
JulAug	20	0
SepOct	18	2
NovDec	18	0

b. Denny Lodge

	presence	**absence**
JanFeb	18	2
MarApr	16	4
MayJun	3	17
JulAug	0	20
SepOct	9	11
NovDec	9	11

c. Overall differences between sites

	presence	**absence**
Brinken	110	7
Denny Lodge	55	67

d. Overall differences between bimonths

	presence	**absence**
JanFeb	38	2
MarApr	36	4
MayJun	17	22
JulAug	20	20
SepOct	27	13
NovDec	27	11

For a direct comparison to be made between the sites, the same species or groups of vegetation had to be present at both sites being compared. Brinken and Denny Lodge had 38 species or groups of vegetation in common with each other, Great Covert and Squabb possessed 29 common species or groups.

Log linear analysis was carried out on all three sets of data using forward selection[5]. Initially models with the two-way interaction terms with presence/absence (*i.e.* site*presence/absence and bimonth*presence/absence) were investigated, followed by the three-way interaction site*bimonth*presence/absence.

Log linear analysis using two-way interactions; site*presence/absence and bimonth* presence/absence, produced significant results for most of the plant species tested, for either or both of the interactions. Tables 2.4-2.5 show that nearly all the species or groups of vegetation showed highly significant interactions for their presence/absence scores and **bimonth**.

The occurrence of many plant species and groups in the diet were influenced by the species of deer and the site. When considering **site** as the independent variable for fallow deer, only 14 out of the 36 plant types tested were significant at the $P \leq 0.001$ level. Nine out of the 29 plant species or groups differed between the two roe sites. The model using the three-way interaction: site by bimonth by presence/absence produced significant results for many of the plant species and groups some of which are evident in Figures 2.8-2.9.

The six most significant species/groups ($P \leq 0.001$) with the largest LR χ^2 (likelihood ratio chi squared) values from Tables 2.4-2.5 are shown in Figures 2.8-2.9, where the percentage occurrence was calculated from the presence/absence data, each will be considered separately.

2.3.2.1 Fallow deer - Brinken vs Denny Lodge

From Figures 2.8a-f it is clear that the majority of the significant effects of site are being driven by Brinken where the occurrence in the diet of most species (except Pine) was higher than at Denny Lodge. Many of the species occurred in at least 60% of the samples at Brinken.

At Brinken **Bramble** occurred in all bimonths in over 60% of the samples whereas at Denny Lodge it was found in just over 40% at its highest (JanFeb). **Pine** was very prevalent at Denny Lodge occurring in nearly all samples (≥90%). At Brinken it occurred mostly in the winter months

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[5] selection method specifies how independent variables are entered into the analysis, forward selection enters the variables in the block one at a time to construct the regression model.

Table 2.4 Log linear analysis for the effects of site and time of year on the occurrence of plant species and groups in the diet of **Fallow deer** (Brinken vs Denny Lodge).

Legend for Tables 2.4-2.5: site = site*presence/absence; bimonth = bimonth*presence/absence; site*bimonth = site*bimonth*presence/absence; LR χ^2 = likelihood ratio chi square; P = probability level; df = degrees of freedom; shaded cells indicate those species showing the highest level of significance particularly for site; cells left blank indicate no significant effect was found.

	site (df = 1)		**bimonth** (df = 5)		**site*bimonth** (df = 5)	
	LR χ^2	**P≤**	**LR χ^2**	**P≤**	**LR χ^2**	**P≤**
Agrostis setacea			140.67	0.0001		
Agrostis tenuis			30.7	0.0001		
Beech			62.117	0.0001		
Birch			86.09	0.0001	38.95	0.0001
Bog Asphodel	13.65	0.001			30.3	0.0001
Brachypodium sylvaticum	44.473	0.0001	72.092	0.0001	19.003	0.01
Bracken			66.187	0.0001	16.828	0.005
Bramble	99.965	0.0001			19.742	0.005
Bromus erectus			46.821	0.0001		
Calluna vulgaris			58.483	0.0001		
Carex spp.	4.009	0.05	57.142	0.0001		
Coarse grass	39.281	0.0001	120.833	0.0001		
Dactylis glomerulata	44.654	0.0001	54.738	0.0001	14.312	0.05
Deciduous tree	6.498	0.05	77.578	0.0001		
Deschampsia caespitosa	4.582	0.05	81.512	0.0001		
Deschampsia flexuosa	35.433	0.0001	66.752	0.0001	25.911	0.0001
Dicotyledon	7.169	0.005	13.121	0.05		
Erica cinerea	4.426	0.05	13.506	0.05	12.378	0.05
Festuca rubra			48.373	0.0001	12.805	0.05
Herb			74.44	0.0001	68.264	0.0001
Grass			112.431	0.0001		
Holcus lanatus			16.904	0.005	14.563	0.05
Holly	72.537	0.0001	44.122	0.0001	15.807	0.01
Ivy	17.564	0.0001	21.166	0.001		

Juncus spp.	19.747	0.0001	18.961	0.005	19.558	0.01
Lolium perenne	43.312	0.0001	47.728	0.0001	32.521	0.0001
Molinia caerula	16.384	0.0001	66.195	0.0001	20.694	0.001
Monocotyledon	8.577	0.005	45.882	0.0001		
Moss						
Oak	11.454	0.001	136.659	0.0001		
Pine	48.454	0.0001	16.875	0.005		
Sieglingia decumbens			85.048	0.0001	28.372	0.0001
Sphagnum			44.594	0.0001		
Thistle	3.951	0.05	26.99	0.0001	29.866	0.0001
Vaccinium myrtillus	12.306	0.001	14.374	0.05	33.494	0.0001
Yew			24.04	0.001		

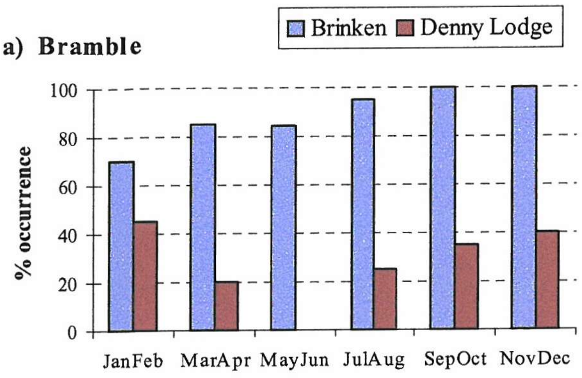
(JanFeb and NovDec) where it was in ≥60% of the samples, there was a clear pattern at Brinken in its occurrence, decreasing through the year and increasing the following winter. At Brinken **Holly** was similar in occurrence to Pine at Denny Lodge, present in nearly all samples all year round, but Denny Lodge showed high occurrences in the early part of the year trailing off as time progressed and picking up only slightly in the last two bimonths.

The grasses, ***Brachypodium sylvaticum*, *Dactylis glomerata*** and ***Lolium perenne*** occurred mostly at Brinken, the latter was relatively rare in the diet at Denny Lodge. The reason for this apparent omission from the diet at Denny Lodge may be because many grasses were unidentifiable down to species level and were therefore grouped into Coarse grass and Grass (Appendix 2). Coarse grass showed a significant effect of site (Table 2.4) where Denny Lodge displayed the highest occurrence.

Of these six species and groups all except Bramble showed significant effects of bimonth, demonstrating that occurrence in the diet fluctuates over the year. All, except Pine, showed a significant interaction between site and bimonth, indicating that not only did the occurrence fluctuate over the year but the manner (pattern) in which it did so was different at the two sites. These results are of more interest than the overall effect of bimonth, where the data were pooled in the analysis from both sites. There is clearly a difference between the two sites in terms of the distribution over the year which is apparently masked by the effect of site. It should however be noted that the significance levels of these findings were lower ($0.005<P<0.05$) than for site and bimonth separately

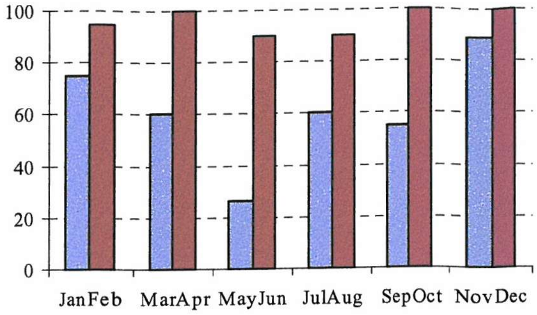
Figures 2.8a-f Plant species showing the greatest differences between the two **fallow** sites in terms of their percentage occurrence in the diet.

a) Bramble



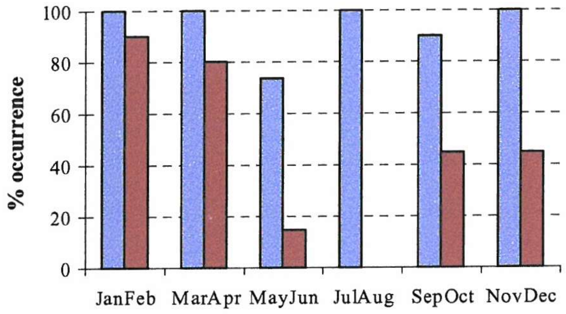
site: LRχ^2_1 = 99.965 P<0.001
bimonth: NS
site*bimonth: LRχ^2_5 = 19.742 P<0.005

b) Pine



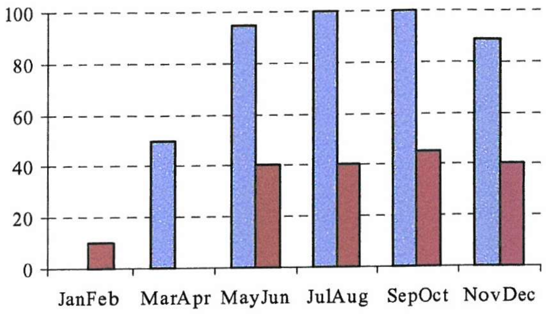
site: LRχ^2_1 = 48.454 P<0.001
bimonth: LRχ^2_5 = 16.875 P<0.005
site*bimonth: NS

c) Holly



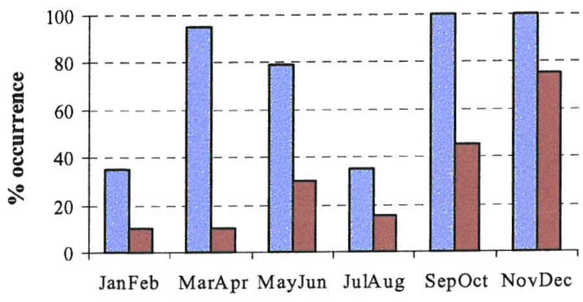
site: LRχ^2_1 = 75.537 P<0.001
bimonth: LRχ^2_5 = 44.122 P<0.001
site*bimonth: LRχ^2_5 = 15.807 P<0.01

d) *Brachypodium sylvaticum*



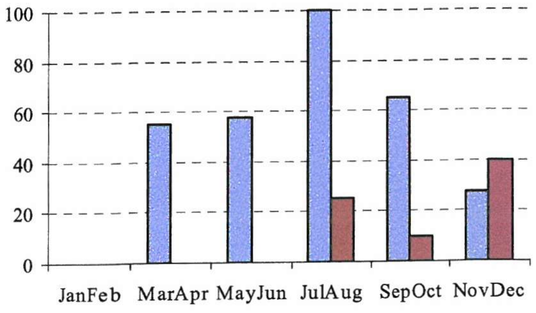
site: LRχ^2_1 = 44.473 P<0.001
bimonth: LRχ^2_5 = 72.092 P<0.001
site*bimonth: LRχ^2_5 = 19 P<0.01

e) *Dactylis glomerata*



site: LRχ^2_1 = 44.654 P<0.001
bimonth: LRχ^2_5 = 54.738 P<0.001
site*bimonth: LRχ^2_5 = 14.312 P<0.05

f) *Lolium perenne*



site: LRχ^2_1 = 43.312 P<0.001
bimonth: LRχ^2_5 = 47.728 P<0.001
site*bimonth: LRχ^2_5 = 32.521 P<0.001

Table 2.5 Log linear analysis for the effects of site and time of year on the occurrence of plant species and groups in the diet of **Roe deer (Great Covert vs Squabb)**. Legend see Table 2.4.

	site (df = 1)		**bimonth** (df = 5)		**site*bimonth** (df = 5)	
	LR χ^2	**P≤**	**LR χ^2**	**P≤**	**LR χ^2**	**P≤**
Alder			79.86	0.0001		
Agrostis setacea			21.669	0.001		
Birch	11.974	0.001	95.94	0.0001		
Blechnum spicant			63.362	0.0001	42.717	0.0001
Bracken			24.545	0.001	56.608	0.0001
Bramble	44.861	0.0001	31.315	0.0001		
Carex spp.	66.033	0.0001			21.375	0.001
Cereal			57.916	0.0001	12.576	0.05
Dactylis glomerulata	19.654	0.0001	51.525	0.0001		
Deciduous tree	11.756	0.05	24.829	0.0001	62.032	0.0001
Deschampsia flexuosa			11.634	0.05	13.653	0.05
Dicotyledon						
Douglas fir	135.791	0.0001			49.755	0.0001
Dryopteris pseudomas	5.186	0.05	56.015	0.0001	12.446	0.05
Herb	34.584	0.0001	57.769	0.0001	12.078	0.05
Grass			13.419	0.05	13.394	0.05
Holcus lanatus			12.923	0.05		
Ivy	54.6	0.0001	28.05	0.0001		
Juncus spp.	6.017	0.05	27.579	0.0001	15.752	0.01
Larch	9.374	0.005	54.18	0.0001		
Lolium perenne	12.363	0.001	31.315	0.0001		
Monocotyledon	4.042	0.05				
Moss			20.71	0.001	29.995	0.0001
Oak			105.577	0.0001		
Pine			39.525	0.0001	35.893	0.0001
Ragwort			11.427	0.05	14.76	0.05
Sweet chestnut			11.708	0.05	21.587	0.001
Thistle	6.701	0.01	12.298	0.05	14.293	0.05
Western hemlock	117.055	0.0001			15.225	0.01

2.3.2.2 Roe deer - Great Covert vs Squabb

Figure 2.9a-f show the five plant species and one group that varied the greatest between the two roe sites. **Bramble** occurred in every sample throughout the year at Squabb, suggesting that every deer ate Bramble all year. At Great Covert, in sharp contrast, the deer followed a pattern that increased through the year, peaking in late summer/autumn. At Squabb **Ivy** occurred in most samples over the year (≥80%), at Great Covert the value was similar in the early part of the year but decreased greatly after spring with no real recovery over the remainder of the year. ***Carex* spp.** occurred in most samples at Squabb (≥70%) whereas at Great Covert it was never in more than 60%. At Squabb there was a slight dip in MayJun in contrast to Great Covert where there was a peak in occurrence during this particular bimonth. The deer at Great Covert had a very high consumption of **Western hemlock** in their diet, all but one bimonth (JulAug) showing 100% occurrence. Squabb on the other hand showed 50% occurrence or less throughout the year.

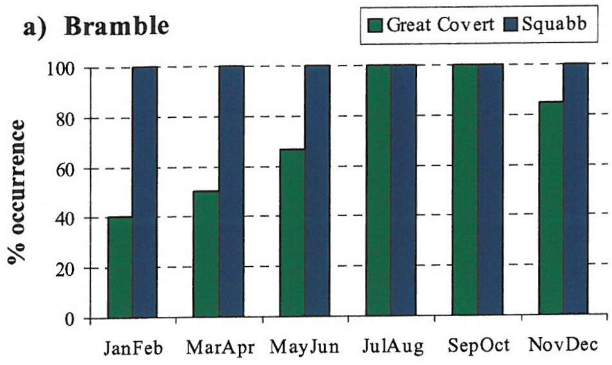
Herbs showed a similar pattern at both sites but in differing amounts. There was a steady increase into the summer after which there was a decline in occurrence at both Great Covert and Squabb, but particularly the former. **Douglas fir** only occurred in the diet at Squabb in JulAug and SepOct and then only at relatively small levels. At Great Covert, with the exception of JulAug, it was present in ≥80% of samples.

Western hemlock, Douglas fir and *Carex* spp. did not show significant differences between the bimonths. *Carex* spp., as already mentioned, displayed opposite trends at the two sites explaining the non significant effect of bimonth but the highly significant ($P<0.001$) interaction between site and bimonth. Douglas fir too, showed a highly significant interaction (site*bimonth) but was largely driven by the absence of this species at Squabb over much of the year. Western hemlock showed a significant effect of the interaction term but to a lesser extent ($P<0.01$), as this species was present in the diet at Squabb but occurred in fewer deer.

The three remaining species and groups showed significant effects of bimonth. Bramble and Ivy showed a non significant result for the interaction between site and bimonth indicating that for these species the pattern of occurrence over the year was the same at both sites albeit at different levels. Only Herb showed a significant effect of bimonth and the interaction term, the latter being of a relatively low probability level ($P<0.05$) and presumably driven by the lack of this group in the early and late parts of the year at Great Covert.

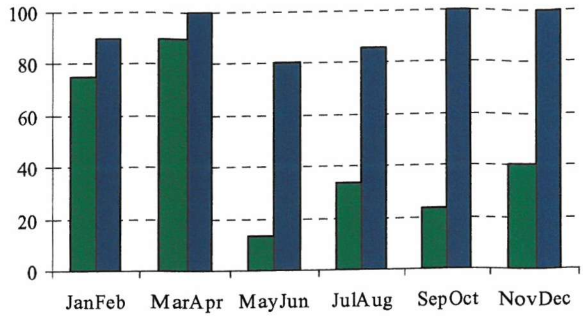
Figures 2.9a-f Plant species showing the greatest differences between the two **roe** sites in terms of their percentage occurrence in the diet.

a) **Bramble**



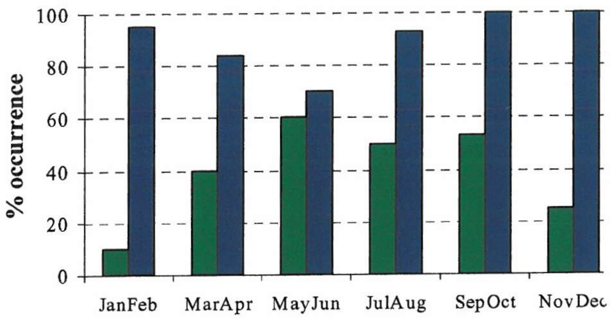
site: LRχ^2_1 = 44.861 $P<0.001$
bimonth: LRχ^2_5 = 31.315 $P<0.001$
site*bimonth: NS

b) **Ivy**



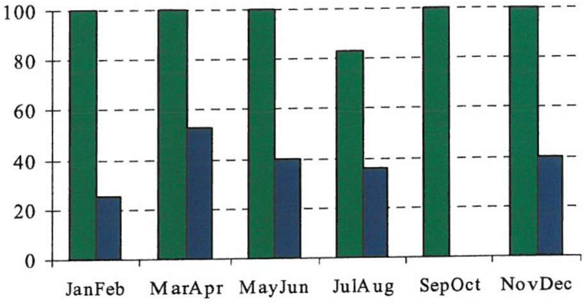
site: LRχ^2_1 = 54.6 $P<0.001$
bimonth: LRχ^2_5 = 28.05 $P<0.001$
site*bimonth: NS

c) ***Carex* spp.**



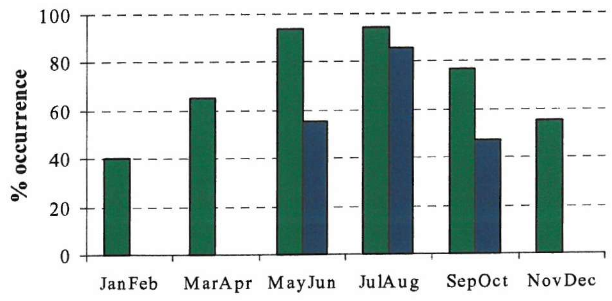
site: LRχ^2_1 = 66.033 $P<0.001$
bimonth: NS
site*bimonth: LRχ^2_5 = 21.375 $P<0.001$

d) **Western hemlock**



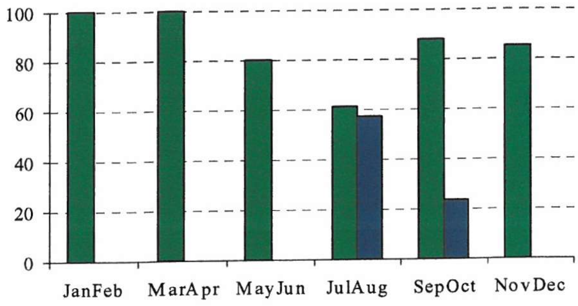
site: LRχ^2_1 = 117.06 $P<0.001$
bimonth: LRχ^2_5 = NS
site*bimonth: LRχ^2_5 = 15.225 $P<0.01$

e) **Herb**



site: LRχ^2_1 = 34.584 $P<0.001$
bimonth: LRχ^2_5 = 57.769 $P<0.001$
site*bimonth: LRχ^2_5 = 12.078 $P<0.05$

f) **Douglas fir**



site: LRχ^2_1 = 135.791 $P<0.001$
bimonth: LRχ^2_5 = NS
site*bimonth: LRχ^2_5 = 49.755 $P<0.001$

2.3.3 Volume (proportion) of plant species in the diet

The volume of plant species in the diet has been determined in two ways. Firstly the data will be described in terms of how other researchers have described the diet of fallow and roe deer (Section 2.3.3.1). This will allow comparisons to be made between different studies placing the current study into context. It will also help examine the reliability of the faecal analysis technique: if most of the plant species eaten at the study sites are the same as those eaten in other research studies, and the seasonal variation is the same or similar, then the faecal analysis technique will have yielded reliable results, thus giving confidence in its use to determine diet selection (Chapter 5).

Secondly, each species will be statistically analysed (Section 2.3.3.2) to investigate differences between the two fallow sites (Brinken vs Denny Lodge) and the two roe sites (Great Covert vs Squabb). Seasonal variations will also be explored.

2.3.3.1 Description of fallow and roe deer diet

This section deals with each species of deer in turn; fallow deer at Brinken and Denny Lodge; roe deer at Great Covert and Squabb. For each site the plant groups listed in Appendix 2 and Holly, Bramble and Ivy are described. Groups were used to allow comparison with published data as these are standard groupings. The Monocotyledon group was broken down further and direct comparisons, where possible, were made of the actual plant species.

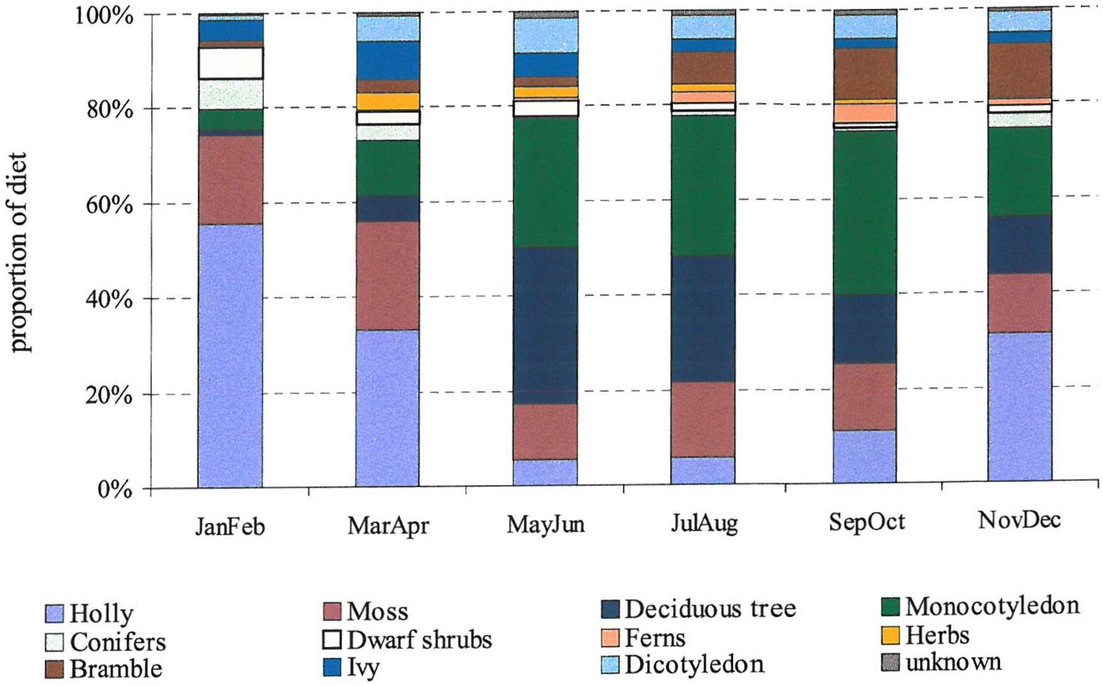
Fallow deer diet

Each plant species or group will be described in detail for each site, Figure 2.10a and 2.10b display the diet composition at Brinken and Denny Lodge. A summary of the main components of the diet at each site is supplied in Table 2.6 and includes data from other researchers (Jackson, 1974, 1977; Putman *et al*, 1993). Discussion of the current data in terms of these other studies can be found in Section 2.4.4.1.

Holly - at Brinken the plant eaten mostly through the winter months (JanFeb and NovDec) was Holly and this persisted into spring (MarApr). Through the summer months its intake was reduced (Figure 2.10a) but it still remained important. In contrast, the fallow deer at Denny Lodge did not eat much Holly. It was only present in the diet in JanFeb (10%) after which time there was a decrease to virtually nothing in the summer months and it did not recover the following winter (Figure 2.10b).

Figures 2.10a-b Diet composition of fallow deer

a) Brinken



b) Denny Lodge

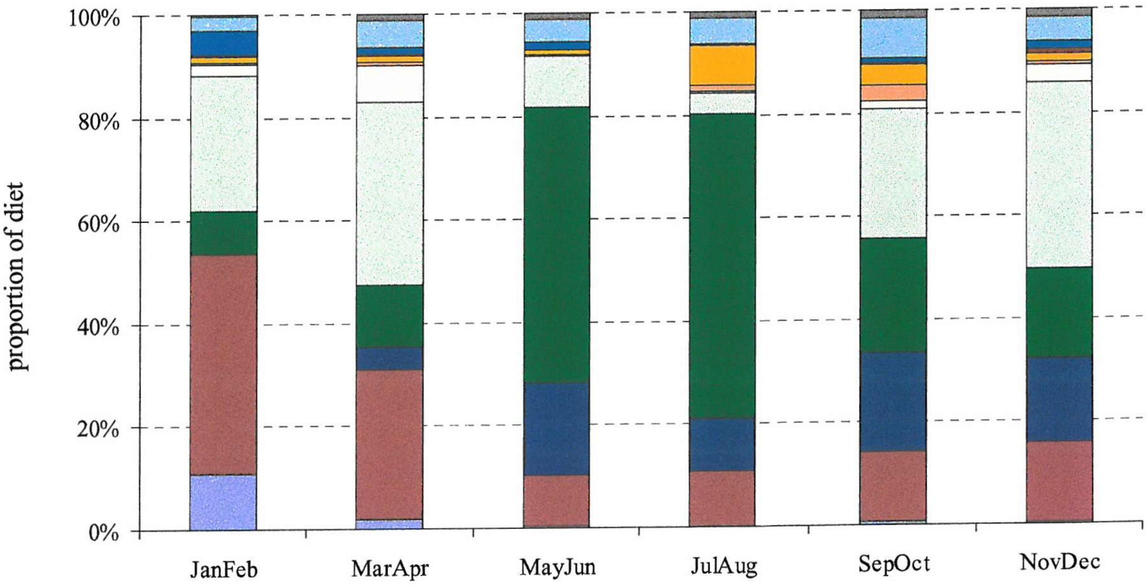


Table 2.6 Main components (in order of importance) of diet for fallow deer over the year using plant groups listed in Appendix 2 and Holly, Bramble, Ivy, *Calluna* sp. and *Erica* spp. Legend: Dshrubs = dwarf shrubs; Dtree = deciduous tree; Monocot = Monocotyledon; Jackson's Graminids = grasses; Putman *et al*'s Moss = *Polytrichum* spp.

	JanFeb		**MarApr**		**MayJun**		**JulAug**		**SepOct**		**NovDec**	
Brinken	Holly Moss Dshrubs Conifers		Holly Moss Total Monocot Dtree		Dtree Total Monocot Moss Holly		Total Monocot Dtree Moss Bramble Holly		Total Monocot Dtree Moss Holly Bramble		Holly Total Monocot Moss Dtree Bramble	
Denny Lodge	Moss Conifers Holly Total Monocot		Moss Conifers Total Monocot Dshrubs		Total Monocot Dtree Moss Conifers		Total Monocot Moss Dtree Herbs		Conifers Total Monocot Dtree Moss		Conifers Total Monocot Dtree Moss	
Jackson (1974, 1977)	Graminids Dshrubs Bramble Conifers Holly	Dshrubs Graminids Holly Conifers Bramble	Graminids Dshrubs Holly Conifers	Graminids Dshrubs Holly Herbs Dtree	Graminids Dtree Dshrub Herbs			Graminids Herbs Bramble Dtree	Graminids Bramble Herbs Dtree	Graminids Bramble Dtree	Graminids Dshrubs Bramble Dtree Conifers	Graminids Dshrubs Conifers Bramble Holly
Putman *et al* (1993) ***Males***	Grasses	Grasses Holly Moss	Grasses Holly Moss	Grasses Moss Holly	Grasses Dtree	Grasses Dtree	Grasses Dtree	Grasses	Grasses	Grasses Moss	Grasses	Grasses Moss
Putman *et al* (1993) ***Females***	Grasses Moss *Erica* spp.	Grasses Moss Holly Dtree	Grasses Moss Holly	Grasses Moss	Grasses Dtree	Grasses Holly	Grasses	Grasses	Grasses	Grasses Moss	Grasses Moss *Calluna* sp	Grasses Moss *Calluna* sp

N.B. Caldwell *et al* (1983) data were not included as they were reported in terms of seasons as opposed to discrete months and so were not comparable.

Deciduous trees - the intake of leaves from Deciduous trees at Brinken increased as the year progressed peaking in MayJun and remained high in JulAug (Figure 2.10a). In both of these bimonths Deciduous tree consumption accounted for at least a quarter of the entire diet (27-33%). Despite the senescence of leaves and the assumed lack of nutrients they then contained, they were still present in relatively high numbers through autumn and winter. At Denny Lodge, Deciduous trees became apparent in the diet from MarApr and increased in the following bimonth where there was an initial peak (18% of the diet), a second peak occurred in SepOct (19%) (Figure 2.10b). As at Brinken Deciduous tree leaves were still present in NovDec in relatively high numbers.

Monocotyledon - the consumption of Monocotyledons at Brinken (Figure 2.10a) increased as the year progressed and peaked in the late summer (JulAug - 30%) and autumn (SepOct - 35%). When the group was broken down further the three different types of Monocotyledon followed similar patterns of intake, but the Coarse monocotyledons (Appendix 2) were eaten in the highest numbers over most of the year. However, it should be noted that at the beginning of the year in the present study *Juncus* spp. and *Carex* spp. were taken in preference to the grasses. Monocotyledons at Denny Lodge (Figure 2.10b) gradually increased over the beginning of the year and by MayJun and JulAug were the most important forage taken by the deer representing 54% and 59% of the diet respectively. After this time there was a decline into the winter months. Compared to Brinken, *Juncus* spp. and *Carex* spp. featured a little more strongly in the diet of the deer at Denny Lodge, especially in the earlier part of the year but also in the autumn and early winter months.

Conifers - at Brinken, Conifers were present mostly at the beginning of the year in the winter months of JanFeb, their consumption decreased throughout the year until NovDec when intake resumed (Figure 2.10a). Conifers had a more important role in the diet of the deer at Denny Lodge than at Brinken. They were a major component of the diet in the winter, spring and autumn months. In JanFeb their consumption was second only to Moss, in MarApr they formed the main constituent of the diet and this was also the case for SepOct and NovDec (Figure 2.10b).

Dwarf shrubs - this group of plants only appeared to be important at Brinken in JanFeb, after this intake declined (Figure 2.10a). They did not feature very much in the diet of the deer at Denny Lodge except for during the bimonth MarApr (Figure 2.10b) when it was mainly *Calluna vulgaris* that was eaten.

Ferns - at Brinken Ferns only formed a small part of the diet in the late summer (JulAug) and autumn (SepOct) (Figure 2.10a). At Denny Lodge they were mainly only taken in SepOct and then only in small amounts (Figure 2.10b).

Herbs - intake of Herbs at Brinken peaked in MarApr and were present in the diet through MayJun and JulAug (Figure 2.10a). At Denny Lodge they were present at very low levels in MayJun (<1%) but increased to a peak in JulAug (8%) after which time there was a more gradual decline (Figure 2.10b).

Bramble - at Brinken the intake of Bramble increased very gradually through the year, representing over 5% of the diet by JulAug, increasing to over 10% in SepOct and NovDec (Figure 2.10a). In contrast at Denny Lodge it was hardly eaten at all, it did not comprise >1% of the diet in any one bimonth over the entire year.

Ivy - this was present in the diet of the Brinken deer throughout the year but it was mainly found in the first half with a peak intake in MarApr (Figure 2.10a). In contrast to this Ivy was not present throughout the year in any substantial amount at Denny Lodge, it peaked at 5% in JanFeb (Figure 2.10b).

Roe deer diet

Each plant species or group will be described in detail for each site, Figure 2.11a and 2.11b display the diet composition at Great Covert and Squabb. A summary of the main components of the diet at each site is supplied in Table 2.7 and includes data from other researchers (Hosey, 1974, 1981; Henry, 1975; Jackson, 1980; Sharma, 1994). Discussion of the current data in terms of these other studies can be found in Section 2.4.4.2.

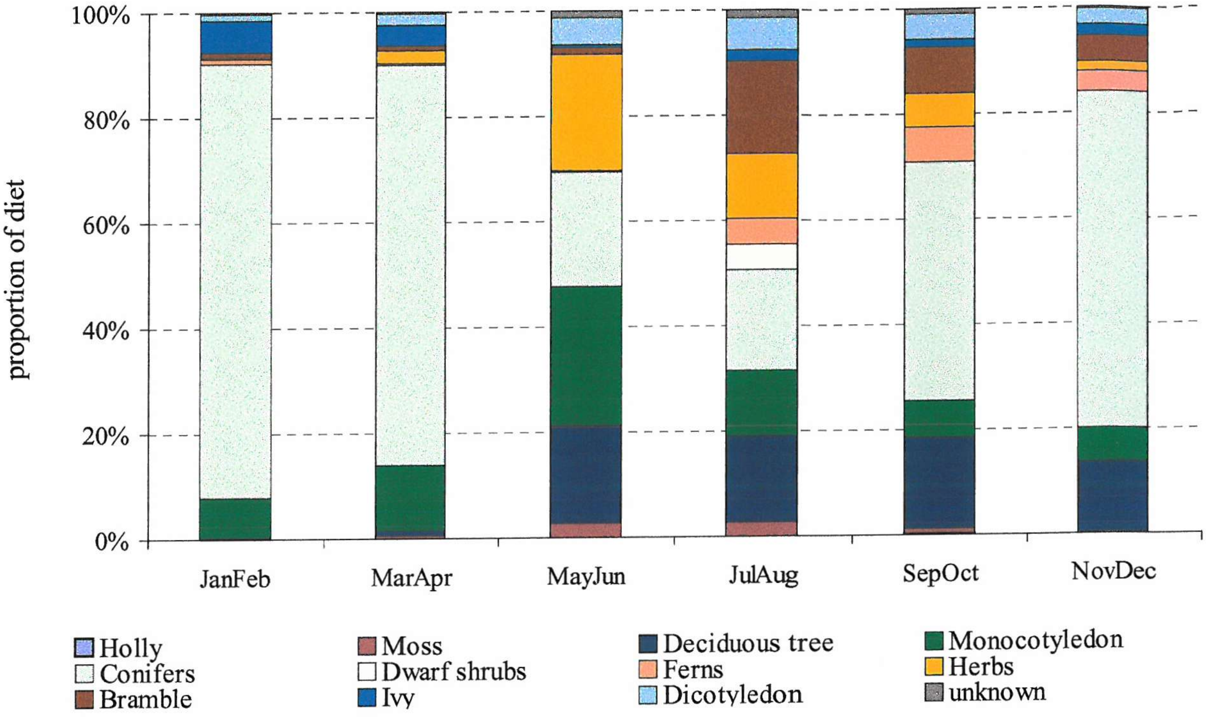
Holly - this species was hardly eaten at all at Great Covert (Figure 2.11a) or Squabb (Figure 2.11b), comprising less than 5% of the diet at both sites, throughout the entire year.

Moss - this, as with the fallow sites was predominantly *Polytrichum* spp. and was only eaten in small amounts with slight increases in consumption in JulAug at Great Covert and Squabb.

Deciduous trees - the leaves of Deciduous trees were eaten from MayJun onwards at Great Covert comprising 13-18% of the diet even into NovDec (13%) (Figure 2.11a). At Squabb leaves were also taken from MayJun (33% of the diet), however, the intake peaked surprisingly late in SepOct (41%) when most of the leaves would be starting to die and fall to the ground (Figure 2.11b). Again, leaves

Figure 2.11a-b Diet composition of roe deer

a) Great Covert



b) Squabb

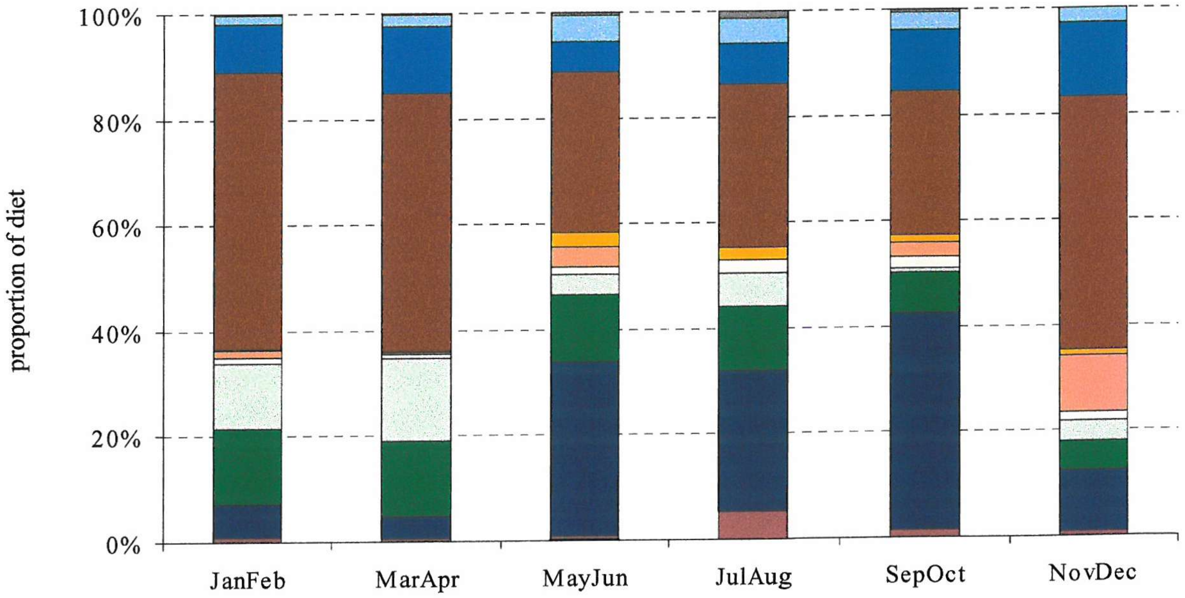


Table 2.7 Main components (in order of importance) of diet for roe deer over the year using plant groups listed in Appendix 2 with Bramble and Ivy. Legend: Dshrubs = dwarf shrubs; Dtree = deciduous tree; Monocot = Monocotyledon

	JanFeb		MarApr		MayJun		JulAug		SepOct		NovDec	
Great Covert	Conifers Total monocot Ivy		Conifers Total monocot		Total monocot Herbs Conifers Dtree		Conifers Bramble Dtree Herbs Total monocot		Conifers Dtree Bramble Total monocot Herbs Ferns		Conifers Dtree Total monocot	
Squabb	Bramble Total monocot Conifers Ivy Dtree		Bramble Conifers Total monocot Ivy		Dtree Bramble Total monocot Ivy		Bramble Dtree Total monocot Ivy Conifers		Dtree Bramble Ivy Total monocot		Bramble Ivy Dtree Ferns Total monocot	
Hosey, 1974, 1981	Bramble Grasses Herbs	Bramble	Bramble Herbs Grasses	Bramble Conifers Herbs	Bramble Grasses Dtree Herbs Conifers	Dtree Bramble	Dtree Bramble Grasses	Bramble Dtree Grasses	Dtree Bramble Herbs	Dtree Bramble Herbs	Bramble Dtree Herbs Ferns	Bramble Herbs Grasses Conifers
Henry, 1975	Dshrubs Conifers Monocot Ferns	Dshrubs Conifers Monocot Ferns	Dshrubs Monocot Conifers	Dshrubs Monocot Conifers	Dshrubs Monocot Conifers	Dshrubs Monocot Conifers Herbs Ferns	Dshrubs Herbs Monocot Ferns	Dshrubs Monocot Ferns Herbs	Dshrubs Ferns Monocot Herbs	Dshrubs Monocot	Dshrubs Monocot Conifers	Dshrubs Conifers Monocot Ferns
Jackson, 1980	Conifers Bramble Ivy Dshrubs Herb	Bramble Conifers Ivy Dshrubs Grasses		Herbs Bramble Dshrubs Dtree Grasses Conifers	Bramble Dtree Herb Grasses Ivy Fern	Bramble Holly Dtree Herb Conifer Grasses	Bramble Herb Dtree Grasses Dshrub			Bramble Dtree Conifers Ivy Grasses		Bramble Dshrubs Herb
Sharma, 1994	Conifers Dtree Grasses Herbs Ferns	Conifers Herbs Grasses Dshrub Dtree Ivy	Conifers Herbs Dtree Dshrub Ferns	Conifers Herbs Dtree Dshrub Grasses	Dtree Conifers Herbs Grasses Bramble	Dtree Conifers Grasses Dshrub Herbs Bramble Fern	Dtree Conifers Grasses Bramble Herbs Fern	Dtree Grasses Ferns Herbs Bramble Conifers Dshrub	Dtree Grasses Ferns Herbs Dshrub Bramble Conifers	Dtree Conifers Grasses Ferns Mosses Herbs Bramble	Dtree Conifers Ferns Dshrub Grasses	Conifers Dtree Grasses Herbs Ferns

were also eaten into and throughout the winter (NovDec and JanFeb).

Monocotyledon - at Great Covert this group showed an increase in intake as the year progressed with a peak in MayJun followed by a steady decline (Figure 2.11a). *Juncus* spp. and *Carex* spp. were taken in higher amounts in MayJun and SepOct. The deer at Squabb ate Monocotyledons in fairly consistent amounts throughout most of the year, decreasing slightly in SepOct and NovDec (Figure 2.11b). The amounts eaten were very similar to those found at Great Covert and also for the published diets, however the peaks of consumption did not coincide. *Juncus* spp. and *Carex* spp. played an even more important role in the diet at Squabb than they did at Great Covert, being present throughout the year. In the latter part of the year, JulAug onwards they were taken in much higher numbers than grasses, particularly in JulAug where they formed 8.5% of the entire diet. Only in MarApr did their consumption go below that of coarse and sweet grasses.

Conifers - these comprised the major dietary component for the deer at Great Covert for most of the year (Figure 2.11a). At Squabb they were moderately important in JanFeb and MarApr (Figure 2.11b), but not to the extent of Great Covert.

Dwarf shrubs - only eaten in any notable amounts in JulAug (Figure 2.11a). At Squabb they were eaten from MayJun through to SepOct but only in small amounts (Figure 2.11b), comprising no more than 2.5% of the diet in any one of these bimonths.

Ferns - eaten only from JulAug onwards and showed a slight peak (6.5%) in SepOct at Great Covert (Figure 2.11a). This increase was also observed at Squabb, in addition there was a considerable increase in NovDec when 11% of the diet was represented by this group (Figure 2.11b).

Herbs - at Great Covert there was a clear peak in the consumption of Herbs in MayJun (22%), after which there was a steady decrease (Figure 2.11a). In contrast at Squabb the intake of this group was very small. There was still the same peak in consumption of Herbs in MayJun, but considerably smaller in size (2.75%), after which there was a similar steady decrease (Figure 2.11b).

Bramble - for the deer at Squabb Bramble was the main component of the diet (Figure 2.11b). It was most important during the winter and also MarApr, over this time it represented approximately 50% of the diet. During the summer and autumn the intake was still very high (~30%) and it remained the major component alongside Deciduous trees. At Great Covert Bramble only became one of the main components of the diet from JulAug (when it peaked; Figure 2.11a).

Ivy - mainly only present in the diet of the deer at Great Covert during JanFeb and MarApr (Figure 2.11a). At Squabb it was eaten at a relatively consistent level throughout the year, however the greatest amounts were taken in the winter, spring and autumn (Figure 2.11b).

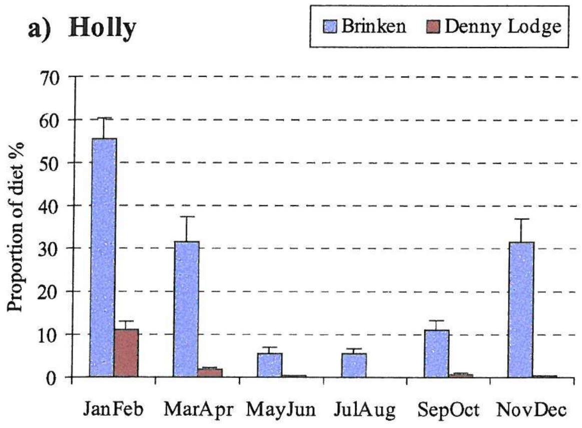
2.3.3.2 Statistical analysis of fallow and roe deer diet based on individual plant species

The volume of each plant species eaten over the year was examined at each site and was found not to be normally distributed. Therefore, the data for all four sites were normalised using an arcsine square root transformation which is acceptable and common for data expressed as proportions (Dytham, 1999). The transformed data were then analysed using the parametric test, Analysis of Variance (ANOVA). In this case ANOVA was testing for significant differences within the fallow sites and the roe sites and also between the bimonths, the two-way interaction term: site*bimonth was also explored.

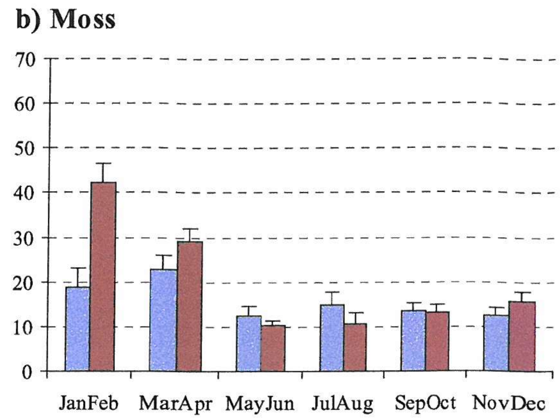
The plant species common to both fallow sites, Brinken and Denny Lodge, were analysed using ANOVA with site and bimonth as the main effects and site*bimonth as the interaction term. Where a significant interaction, site*bimonth, was found the main effects of site and bimonth would also be significant, in such cases only the interaction terms were reported. Of the species that occurred in ≥5% of the diet in any one bimonth at both sites (see Tables A4.1-2, Appendix 4) all except Deciduous tree showed significant interaction between site and bimonth ($F_{5,225} = 3.1\text{-}21.21$, $P<0.01\text{-}0.001$). Figures 2.12a-f show these effects. Holly was eaten mostly at Brinken; Moss only slightly more at Denny Lodge; Oak more at Brinken; Pine more at Denny Lodge; Deciduous tree similar at both; Monocotyledon more at Denny Lodge. The problem with the groups (Deciduous tree and Monocotyledon) was that these were made up of different species some of which may have been identified as separate species at one site and not at another. Therefore the results of the grouped species and the individual species that make up the groups should be interpreted with caution.

Species that represented ≥2% of the diet in any one bimonth at both sites (see Tables A4.1-2, Appendix 4) were also considered as some of these represented ≥5% of the diet for one of the sites and were therefore of importance in the overall diet. Ivy, Grass, *Carex* spp. and *Agrostis setacea* showed significant interaction terms ($F_{5,225} = 4.82\text{-}12.31$, $P<0.001$). Only the main effects of site and bimonth were significant for *Juncus* spp. (site: $F_{1,225} = 38.19$, $P<0.001$; bimonth: $F_{5,225} = 25.19$, $P<0.001$). Bracken, however, was the only species that was not significantly different between the sites; fallow deer at both sites ate it in the same amounts. Moreover, they ate it in the same amounts

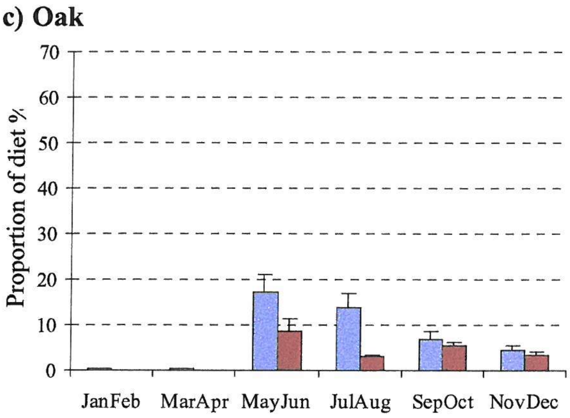
Figure 2.12a-f Relationship between **Brinken and Denny Lodge** for plant species or groups that represent ≥5% of diet in any one bimonth at both sites (mean proportion ± standard error).


a) Holly

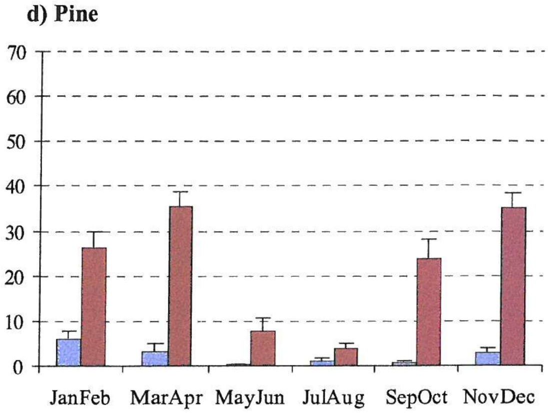
ANOVA: site*bimonth: $F_{5,225}$ =10.03, P<0.001


b) Moss

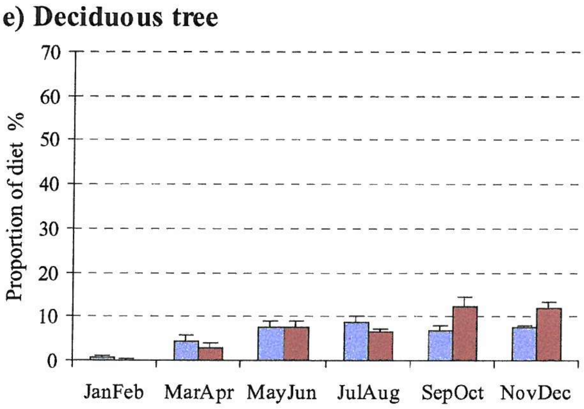
site*bimonth: $F_{5,225}$ =6.65, P<0.001


c) Oak

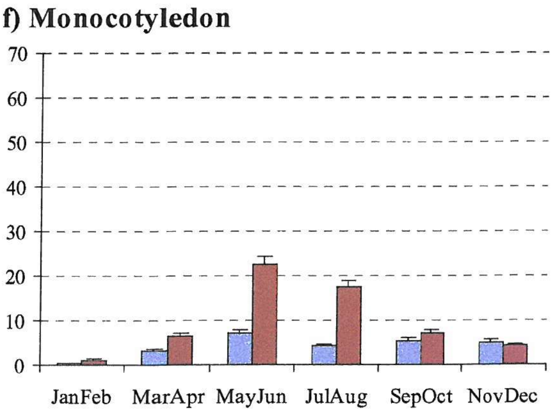
ANOVA: site*bimonth: $F_{5,225}$ =3.1, P<0.01


d) Pine

site*bimonth: $F_{5,225}$ =11.51, P<0.001


e) Deciduous tree

ANOVA: site*bimonth: $F_{5,225}$ =4.34, P<0.001


f) Monocotyledon

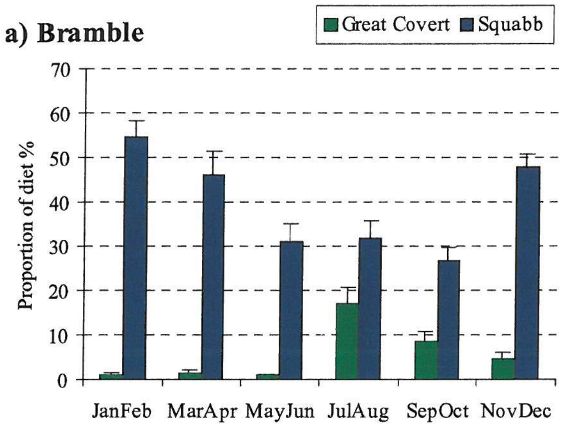
site*bimonth: $F_{5,225}$ =21.21, P<0.001

at the same times of year, indicated by the lack of significant interaction between site and bimonth

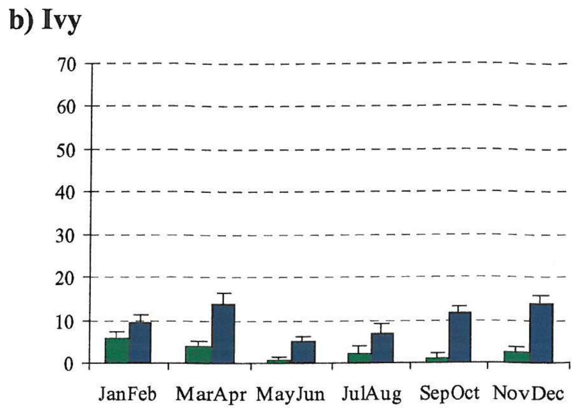
The plant species common to the roe sites, Great Covert and Squabb, were analysed using ANOVA in the same way as the fallow deer sites. All of the species that occurred in ≥5% of the diet in any one bimonth at both sites (Tables A4.3-4, Appendix 4) showed significant interactions between site and bimonth ($F_{5,203} = 2.87\text{-}17.16$, $P<0.05\text{-}0.001$). Therefore the deer at each site ate these species or groups in different amounts, which also varied over the year. Figures 2.13a-e show these findings clearly.

Bramble was eaten mostly at Squabb and in a reverse pattern to Great Covert; Ivy more at Squabb; Deciduous tree (group) was eaten slightly more at Squabb; Monocotyledon was eaten mostly at Great Covert; Oak slightly more at Squabb. Of the six species that were found as ≥2% of the diet in any one bimonth at both sites (Tables A4.3-4, Appendix 4), all produced significant interactions between site and bimonth ($F_{5,203} = 3.14\text{-}16.81$, $P<0.05\text{-}0.001$), although for Moss and *Carex* spp., the probability was lower ($P<0.05$ and $P<0.01$, respectively).

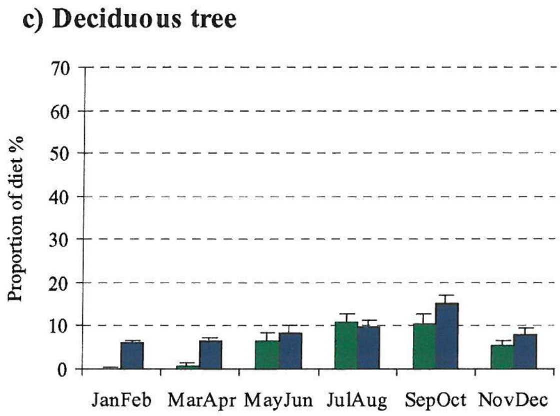
Figure 2.13a-e Relationship between **Great Covert and Squabb** for plant species or groups that represent ≥5% of diet in any one bimonth at both sites (mean proportion ± standard error).


a) Bramble

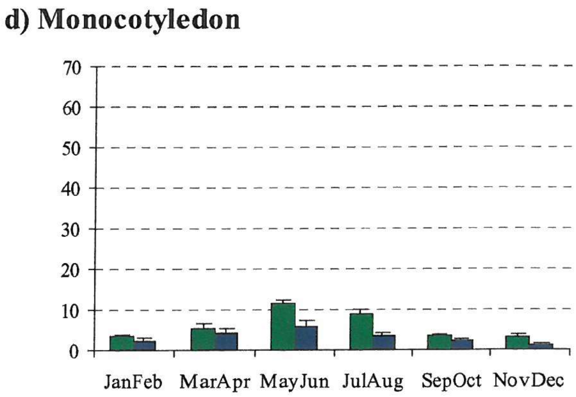
ANOVA: site*bimonth: $F_{5,225} = 17.16$, $P < 0.001$


b) Ivy

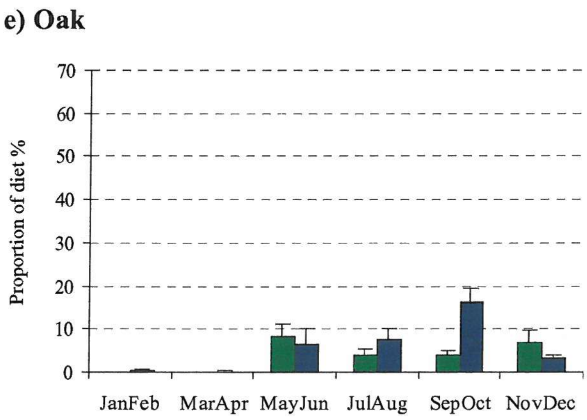
site*bimonth: $F_{5,225} = 3.46$, $P < 0.005$


c) Deciduous tree

ANOVA: site*bimonth: $F_{5,225} = 5.019$, $P < 0.001$


d) Monocotyledon

site*bimonth: $F_{5,225} = 2.73$, $P < 0.05$


e) Oak

ANOVA: site*bimonth: $F_{5,225} = 2.87$, $P < 0.05$

2.4 DISCUSSION

The discussion first addresses limitations of the methodology, mainly by critiquing the faecal analysis technique (Section 2.4.1.1). This is necessary as there is so much controversy about this method of analysis. It will then go on to discuss the results from the data analysis of the faecal samples: Number of plant species eaten (Section 2.4.2); Occurrence of plant species in the diet (Section 2.4.3); Volume of plant species eaten (Section 2.4.4). The latter section will compare the study data with published accounts and assess the reliability of faecal analysis technique for fallow (Section 2.4.4.1) and roe deer (Section 2.4.4.2), in addition to a discussion of the statistical analysis of individual plant species (Section 2.4.4.3).

2.4.1 Limitations of methodology

One of the main limitations with this study was the small number of study sites used for both species of deer, alternatives for which were discussed in Section 2.2.1. The choice of study sites suggested that the effect of competition between the two species was alleviated, a distinct advantage. Roe deer have been suggested as avoiding competition with sympatric species because of their diet selection, but in fact they do suffer from feeding competition (Duncan *et al*, 1998). Although the fallow and roe deer in the current study were not co-existing, the effects of competition could still be being endured by the latter, having been potentially 'pushed out' of more favourable habitats by the former. Despite this being a possibility with the roe study sites the measured diet composition compared well with published accounts, suggesting that the study sites were suitable. Therefore, although competition cannot be ruled out completely, it is believed that this study was not seriously affected by it.

2.4.1.1 Critique of faecal analysis methodology

This study used the numbers of plant fragments per species as a quantitative measure of diet composition. This has been questioned by many researchers (*e.g.* Hercus 1960; Westoby *et al*, 1976; Henry, 1978; Smith and Shadruk 1979; Helle, 1980; Hosey, 1981). One of the main problems is that of **differential fragment size**, thus using the number of counts to represent the relative importance in the diet could be wholly misleading. Two main solutions to this problem have been suggested.

One is to reduce and standardise the size of the fragments by milling or grinding the faecal sample (Sparks and Malechek, 1968) and passing it through a sieve (see Section 2.2.3). The drawback with this is that some plants contain very fragile cuticles (Rogerson *et al* 1976 as cited in Vavra and Holechek, 1980) and clearly milling or grinding would destroy this material. In the present study the samples were ground using a pestle and mortar (Section 2.2.3) thought to be less destructive than mechanical grinding. As Storr (1961) found, most of the fragments in this study were of similar sizes but a few were larger, their presence was probably due to chance as the sample had not been sieved. These could contribute more to the estimation within the diet than the others but Storr (1961) suggested that with enough replicates and pooling of data (as in the current study) the larger plant fragments would not affect the mean proportions of each plant species. This would be practical if larger fragments were not always the same species of plant.

In the present study sieving was not practical due to time and large numbers of samples. The grinding of the samples with a pestle and mortar was found to give identifiable fragments of reasonably similar sizes, and so sieving was considered unnecessary.

The second solution is to not only count the number of fragments for each plant species but to also measure their area using a graticule eyepiece. Storr (1961) states that relative area is of no use and uses the area measured to calculate a dry weight for each species which can be compared. He questions whether this reflects the proportion by weight of the various species ingested and goes on to say that this assumes the digestion of epidermal tissue is same in all perennial plants. Thus, this method is also not without its problems. Stewart (1967) states that analysis by area is very time consuming and there are problems with irregular shapes. Using area has still shown significant differences between the results of faecal analysis and exactly what was ingested but it is an improvement on using fragment counts (Stewart, 1967).

Introducing a correction factor to account for differing fragment size was explored by Stewart (1967). He decided that there would have to be a correction factor specific to each species of plant, each species of animal (he found significant differences in mean fragment size between Thomson, *Gazella thomsoni* and Grants gazelles, *G. granti*) and an animal's age; altogether, too many variables to make correction factors practical.

Another significant problem with using faecal analysis is **differential digestibility**. Plant species are digested in the gut to a lesser or greater degree than others, this means that digestion can have a significant effect on what is found in the faeces. Investigations into this have usually been carried out on captive deer using feeding trials, measuring the relationship between what was ingested and what was egested (*e.g.* Bissel *et al*, 1955; Dietz *et al*, 1962; Drodz, 1979). Using the data from such studies correction factors for differential digestibility have been formulated. The use of feeding trials to obtain correction factors can be complex and expensive. Alternatives have been described by Putman (1984), *ruminal-rectal calibration*: looking at faecal and stomach samples from the same animal (sika deer: Mann, 1983); *actual digestibility*: experimentally derived digestibility usually *in vitro* (Section 4.2.7).

Putman (1984) states that few plant species have constant digestibility and the digestibility of one plant species will vary according to what else the animal has eaten, *i.e.* what is in the mixture. The digestibility of a plant fed on its own is very different to when it forms a small proportion of the diet. This has been shown by Milne *et al* (1978) when looking at the consumption of heather by red deer and Scottish hill sheep. They found that the gut and its flora adapted to the major constituent. This adaptation can take some time which, as Putman (1984) points out, can be a problem when conducting feeding trials. Overall, the digestibility of one forage species varies depending on the mixture and also the recent dietary history. Therefore it is difficult to arrive at true correction factors as to do so would mean that an infinite number of diet compositions would have to be tested. Also it would be impossible to test the accuracy of any of the correction factors, derived by whatever method, as a control diet could not be established (Putman, 1984).

Hansen *et al* (1973), working on sheep, cattle and bison, state that correction factors are not required to account for differences in the digestibility of different plants. Their data suggest that there is no significant difference between plant fragments in oesophageal samples and faecal samples. Johnson and Wofford (1983) agree with Hansen *et al* (1973) and Todd and Hansen (1973), stating that digestibility does not significantly alter botanical composition. However they go on to say that it does significantly affect the discernability but the impact will rarely cause significant overall alterations to diet estimates. They conclude that estimates of the relative importance of different plants will be reasonably accurate. For these reasons these authors advocate the use of faecal analysis to estimate diets.

Holechek (1982) sums up the overall general feeling in this field when he stated that faecal analysis should be interpreted with caution. From his study investigating sample preparation techniques, he recommends care should be taken particularly for samples high in woody plant material. Other authors have focussed on the over- and underestimation of specific plant groups. Holechek and Valdez (1985) state that faecal analysis has limited value in estimating mule deer diets that consume significant but variable quantities of stemmy material from shrubs. Shrubs have lower proportions of identifiable epidermal material per unit weight than grasses and forbs (Zyznar and Urness, 1969; Westoby *et al*, 1976). Westoby *et al* (1976), with Vavra *et al* (1978) and McInnis *et al* (1983) in agreement, found that as a result grasses are overestimated and forbs and shrubs are underestimated. Holechek and Valdez (1985) say such generalisations can not be made, but they do say that shrubs consisting of a lot of stemmy material are severely underestimated particularly when they occur with grasses and forbs, their findings were consistent with Zyznar and Urness (1969) and Gill *et al* (1983). Anthony and Smith (1974) found that herbaceous species were digested more than deciduous and evergreen species thereby giving a biassed results. Overall Westoby *et al* (1976) conclude that there is a tendency to underestimate or miss material present in small amounts (be that as a result of the digestive process or simply ingested in small quantities), and to overestimate the more common taxa.

Despite problems with under- and overestimation, Stewart (1967) states that as long as there is consistency it is possible to compare the proportions of the same grasses (in the case of his study) eaten at different times or in different places by individuals of the same species. Therefore this suggests that in the present study comparisons can be made between months and between sites, but it does imply that comparing the different species of deer may not be reliable. Although fallow and roe deer are both ruminants they do possess very different digestive systems (Section 1.2.4). However, Stewart (1967) goes on to say that grasses are not only digested to different extents by any one animal, but also different animals (within a species) may digest the same grass to different extents, thereby making interspecies comparisons no less reliable than intraspecific comparisons. The digestibility of herbage is influenced only very slightly by animal characteristics and is primarily determined by herbage characteristics (Ivins, 1960, as cited in Stewart 1967).

The overriding factor with the faecal analysis in the current study was the vast numbers of samples that required analysis, time was the critical limiting factor in determining and justifying the preparation and data collection methods used. Stewart (1967) concluded that although using correction factors, fragment area, etc. may be practical for an intensive study involving only a few plants and animal species, it must be accepted that most studies must be limited to obtaining quantitative data based on frequencies of fragments despite the inherent problems. However, once frequencies have been obtained, data on the number and occurrence of species (Sparks and Malechek 1969; Stewart and Stewart, 1970) can be derived. Myers and Vaughan (1964) looking at the diet of Pocket Gophers (*e.g. Geomys* spp.), used the same method of recording plant fragments as the current study, using counts which were converted to proportions of the diet and also occurrence of species. However, it is still important to treat the mean numbers of fragments recorded with caution because of differential digestibility, but Stewart and Stewart (1970) state that if large differences are revealed using frequencies, they are probably meaningful.

From the literature it is evident that microhistological analysis of faecal material is still fraught with problems and limitations for the researcher, and in the end methodological choice is down to the particular criteria imposed by the individual set of circumstances surrounding a study. Where possible the 'guidelines' should be adhered to but various authors have shown that it is satisfactory to forego particular aspects of the analysis, as long as the problems these may impose are recognised and understood. In the current study the problem of differential fragment size was minimised by grinding the samples. Correction factors for differential digestibility appear to be misleading, time consuming and overall their necessity is questionable. In light of this and the lack of sample material in most cases for digestibility analysis, correction factors were not used in this study.

Faecal analysis was by far the best technique for this study and each problem has been considered and discussed. It is believed that all possible precautions were taken to minimise errors and create as robust an analysis as possible within the limitations.

2.4.2 Number of plant species eaten

Brinken showed the highest number of species eaten (mean = 16.29); the ANOVA and subsequent Duncan multiple range test indicated that this site was significantly different to the other fallow site (Denny Lodge) and the two roe sites (Great Covert and Squabb). Denny Lodge displayed the second highest number of species eaten (mean = 12.94); the ANOVA and multiple range test distinguished this site from the two roe sites and, as mentioned above, was significantly different to Brinken. Great Covert and Squabb displayed similar means, the ANOVA and multiple range test suggested that these were not significantly different from one another, but as stated, were different to both fallow sites.

The bimonths with the highest numbers of species or groups eaten by the deer were MayJun, JulAug and SepOct for Brinken and Great Covert, and for Squabb the latter two bimonths showed the highest values. JulAug and SepOct were found to be significantly different to the other bimonths. Denny Lodge, however, showed a steady increase in numbers of plant species eaten over the year the reason for this is unclear. For all sites the lowest mean number of species taken was in JanFeb (means: Brinken = 9.3; Denny Lodge = 9; Great Covert = 8.25; Squabb = 8.9); this bimonth was significantly different to all the other bimonths.

In spring (Mar - May) there were more plants than in the winter months simply as more plants start to grow at this time; they are young, readily digestible and very nutritious (see Dietz *et al*, 1958; Dietz *et al*, 1962). The deer were faced with a wider choice hence the increase in numbers of plant species eaten compared to JanFeb (winter). As the year goes on the plants generally become less nutritious (see Dietz *et al* 1958; Dietz *et al*, 1962) and less digestible (Short, 1971) and the deer had to take a wider variety to obtain what they nutritionally required, consequently the numbers of species taken is at its highest in JulAug and SepOct. After this time many plants die and only the hardier species (including evergreens and grasses) remain through the winter thus the numbers of different species eaten decline as the deer were forced to take fewer. There is a strong suggestion that the numbers of plant species eaten may be closely linked to availability this will be investigated in Chapter 3.

2.4.3 Occurrence of plant species eaten

Overall the two fallow sites showed the greatest differences in the occurrence of plant species and groups. This was demonstrated by the high number of significant differences between sites for vegetation ranging from grasses to conifers. It is unlikely that the differences were due simply to the availability of plants (Section 3.3.3), as occurrence in the diet is not measuring the quantity but simply presence or absence of a species in the diet. Therefore a species could be eaten by many deer in small amounts at one site perhaps because of its low availability, and eaten by many deer in large amounts because of its abundance at the other site and still the occurrence data for each would be the same, *i.e.* present in the diet of many deer. Availability may have some influence as if a plant species is abundant more deer are likely to come into contact with it and therefore the occurrence in the diet will be high. However, this is difficult to unravel in this particular analysis and will be considered further in Chapter 3.

Many of the plants (67%) which showed significant effects of site also proved significant for the interaction between site and bimonth. This indicates that the plants occurred in the diet of the two populations of fallow deer not only in differing amounts (*i.e.* numbers of deer), but also the pattern of occurrence in the diet over the year was different at the two sites. This does not suggest availability as the underlying reason for the differences, as more often than not the same species at each site were available at the same times of year (Section 3.3.3), but that the deer chose to eat them at different times. Presence of a particular species in the diet of one population of deer at a certain time of the year and at a different time of year for another population may therefore be due more to the individual preference of each population.

The two populations of roe deer showed fewer significant differences between each other in terms of the occurrence of vegetation in the diet. Also, less than half of the plant types showed significant interactions between site and bimonth. This implies that for roe deer individual preference, was not operating. Availability of vegetation may be of more relevance to this species of deer (Section 3.3.3).

2.4.4 Volume (or proportion) of plant species eaten

2.4.4.1 Comparison of fallow deer diet with published data

Holly - Jackson (1974, 1977) found that Holly featured as an important part of the diet through the winter months and into March and April as was the case for the deer at Brinken. Putman *et al* (1993) found that males in particular, ate large amounts during February and into March, but consumption by both sexes decreased as the year progressed. This pattern was more similar to that seen at Denny Lodge, peaking in JanFeb.

Moss - featured greatly in the diet of the fallow deer at both sites. Its importance in the diet was reflected in the study by Putman *et al* (1993), both males and females consumed similar amounts to the deer in the present study throughout the year. However, in Jackson's study (1974, 1977) Moss had no place in the diet of the deer. Caldwell *et al* (1983) stated that Moss was eaten accidentally, probably because it was found in trace amounts. The high incidence in the diet of the deer at both study sites suggested that ingestion was not accidental.

Deciduous tree - the pattern of intake at the two fallow sites was parallelled in the studies by Jackson (1974, 1977) and Putman *et al* (1993). The first peak at Denny Lodge corresponded to Jackson's data (1974, 1977) and was of similar magnitude.

Monocotyledon - peaks witnessed at Brinken coincided with the data collected by Putman *et al* (1993), especially for females. For Jackson's data the peaks occurred earlier than this, in April and June and were twice the amount. Putman *et al* found that females took similar amounts of coarse and sweet grasses throughout the year but that the males took slightly more of the sweet grasses. Jackson did not separate out the Monocotyledons in the same way as Putman *et al* and the current study. At Brinken, *Juncus* spp. and *Carex* spp. did not contribute very much to the overall intake of Monocotyledons. The study by Putman *et al*, on the otherhand, showed that over most of the year *Carex* spp. and *Juncus* spp. were important in the diet with the main component being *Juncus bulbosus*, as noted by Jackson. The fallow deer at Denny Lodge conformed more readily to Jackson's data (1974, 1977) than the deer at Brinken. The pattern and importance of this food group in the diet at Denny Lodge is reflected in Jackson's data (1974, 1977) with the peak intake occurring earlier than it did at Brinken (*i.e.* before SepOct). However, in contrast, Jackson's data showed the deer were taking larger amounts in the spring and persisting further into the autumn months than the deer at Denny Lodge. At peak consumption the Monocotyledon diet was made up mostly of coarse grasses as opposed to sweet ones. As mentioned earlier, Putman *et al* found that females took similar amounts of coarse and sweet grasses throughout the year but that the males took slightly more of the sweet grasses, so this pattern was not reflected in the current study. A more consistent consumption of *Juncus* spp. and *Carex* spp. over the year for Denny Lodge corresponds favourably with Putman *et al.*

Conifers - the pattern of intake at both sites was parallelled in Jackson's data (1974, 1977) where he found highest consumption in January, February and December. These plants are a winter component of the diet of fallow deer. Conifers were not recorded in the study by Putman *et al* (1993).

Dwarf shrubs - in the form of *Calluna vulgaris* were of most importance in the early part of the year at Brinken and Denny Lodge. This was also the case in Jackson's data (1974, 1977), he recorded *Calluna vulgaris* as a main constituent of the diet in December, January, February and March. Putman *et al* (1993) recorded low intakes for the majority of the year with a slight increase in September continuing into winter (November and December), during these months the authors noted that females consumed over twice as much as males.

Ferns - lack of Ferns in the diet confirms Jackson's data (1974, 1977) which indicated that fallow deer did not take many at any time of the year. Putman *et al* (1993) found the same was true for male fallow deer but females took *Pteridium aquilinium* in considerably larger amounts in November and December than at other times of the year.

Herbs - consumption peaked early in MarApr at Brinken whereas at Denny Lodge the peak coincided with that found by Jackson (1974, 1977) in JulAug. Putman *et al* (1993) did not record Herbs as a separate group.

Bramble - this was an important component of the diet at Brinken in the latter part of the year, in contrast at Denny Lodge it hardly featured at all. Jackson (1974, 1977) also found this was the case in October and November, but more importantly in January when at Brinken it was hardly taken at all (<1%). Putman *et al* (1993) did not record Bramble separately.

Ivy - Jackson (1974, 1977) found that Ivy was taken consistently over the winter months and peaked in July, by which time at Brinken its consumption had started to abate, although prior to this it had been an important component of the diet especially in MarApr. It was not considered an important element at Denny Lodge.

Reliability of the faecal analysis method for fallow deer

The major difference between the diet of the fallow deer studied by Jackson (1974, 1977), Caldwell *et al* (1983) and also Putman *et al* (1993), with the deer studied here is the importance of grasses (graminids) in the diet. Jackson found that this group of plants constituted the main bulk of the fallow deer's diet forming as much as 75% of the diet in June. This was also the case for both the males and females in the study by Putman *et al*: in the males' diet grasses accounted for 50%-80% of the diet over the year and for the females', 45%-85%. Caldwell *et al* (1983) found that grasses comprised 40% of the diet in autumn and winter. At Denny Lodge they were also important, especially in the summer months, however at Brinken during this time they were less prevalent and other plants had similar weighting in the diet.

As stated earlier (Section 2.4.1.1), grasses have been known to be overestimated in the diet (Westoby *et al*, 1976; Vavra *et al*, 1978; McInnis *et al*, 1983) especially if they occur with stemmy shrubs (Holechek and Valdez, 1985). However, this was certainly not the case at Brinken and Denny Lodge, and as the comparison data were derived from rumen and faecal analysis it can be assumed that the magnitude of those data are correct. The only explanation for what was seen at Brinken and Denny Lodge is the hurricane of January 1990. This storm brought down many trees across the New Forest including several at Brinken and Denny Lodge. This made some foods more readily accessible, particularly Holly at Brinken and Conifers at Denny Lodge. These were eaten in larger quantities than considered usual (Jackson, 1974; 1977) and therefore the normally high proportions of Monocotyledons in the diet decreased. However, this is speculative as the diet of these deer was not known prior to the test year nor was it investigated afterwards.

All of the plants species found in the diet of fallow deer at each study site have been found by other authors and apart from the question of the Monocotyledons (grasses), mentioned above, they were all found in similar amounts usually at the same time of year. This being the case it can be assumed that despite all the potential problems encountered with faecal analysis (see Sections 2.1.1 and 2.4.1.1) the precautions taken and assumptions made in this study meant that the analysis yielded results consistent with the published studies, suggesting the current data are robust and useful for further analysis of diet selection (Chapter 5).

2.4.4.2 Comparison of roe deer diet with published data

Holly - lack of Holly in the diet was also the found by Hosey (1974, 1981), Henry (1975, 1978) and Sharma (1994). Jackson (1980) on the other hand found Holly was taken in relatively large amounts in June and hardly at any other time of year.

Moss - low amounts of Moss found in the diet at Great Covert and Squabb were supported by Jackson (1980) who reported that although Moss was available in relatively large amounts, the deer only took trace amounts, Hosey (1974, 1981) found no Moss in the diet. In contrast, Henry's deer (1975, 1978) ate a wide variety of Mosses throughout the year, and although in small amounts, consumption peaked in July and again in October. This latter peak was also noted by Sharma (1994), but the deer ate very little Moss over the rest of the year. A small peak was observed at the study sites in JulAug. It is possible that roe deer ingest Moss accidentally whilst taking other plants especially Monocotyledons and Herbs (particularly JulAug). This has been suggested for fallow deer by Caldwell *et al* (1983) but was rejected for fallow deer in this study (Section 2.4.4.1).

Deciduous trees - these were the main component of the diet for roe deer, especially at Squabb and, as for fallow deer, the leaves were eaten into the winter (NovDec). Deciduous tree leaves played a considerable role in the diet from June until October (Hosey, 1974; 1981) and April to December (Sharma, 1994). Jackson (1980) noted a peak intake in May. Henry's data (1975, 1978), in contrast, shows that the deer took less than 2% of this food type throughout the entire year.

Monocotyledon - MayJun witnessed the highest consumption of Monocotyledons at Great Covert and Squabb, but was still less than that observed by Henry (1975, 1978). Sharma (1994) witnessed a peak intake in August/September of 21% and 19% of the diet respectively. Although they were not eaten in any great quantity, *Juncus* spp. and *Carex* spp. were favoured at both sites particularly from

MayJun to SepOct. Hosey (1974, 1981) also found that the deer did not eat them in any great quantity, but that most were eaten in March. Jackson (1980) reported *Juncus* spp. and *Carex* spp. as being plentiful in the habitat but not present in the diet. In SepOct very little coarse or sweet grasses were eaten, however a large amount of cereal crop was taken.

Conifers - these were of considerable importance at Great Covert. Data from Henry (1975, 1978), Jackson (1980) and Sharma (1994) also demonstrated the importance of this food type showing increased consumption over the same times of year as Great Covert. However, only in Sharma's (1994) study did the deer eat similar proportions (up to 59% in March) compared to the present study. Hosey found a similar pattern of intake but Conifers were not as important in the diet, 10% was the highest amount eaten in December. At Squabb they did not have a defined role in the autumn (SepOct) and early winter (NovDec) this was in contrast to the diet at Great Covert and for Henry (1975, 1977), Jackson (1980) and Sharma (1994).

Dwarf shrubs - these were not important in the diet of the roe in this study apart from in JulAug at Great Covert. Hosey's deer (1974, 1981) did not take any Dwarf shrubs, but Jackson's (1980) and Sharma's (1994) ate them throughout the year peaking in spring (March and April). For the roe deer in Henry's study (1975, 1978) Dwarf shrubs (namely *Calluna vulgaris*) were the main component of the diet in every month and in this case the peak in consumption was from August through to November where it formed over two thirds of the diet.

Ferns - the increase in consumption of Ferns observed at Great Covert and Squabb in SepOct was reflected in Henry (1975, 1978) and Sharma's (1994) data.

Herbs - deer in Hosey's study (1974, 1981) had a peak intake of Herbs in March, Jackson (1980) and Sharma's (1994) deer peaked in April and Henry's (1975, 1978) in July. In the current study the peak occurred in MayJun. Herbs were more important in the diet of the Great Covert deer than those at Squabb and were eaten in similar amounts to the published studies.

Bramble - this was the main dietary component at Squabb as found for Hosey's (1974, 1981) deer. In contrast the data from Great Covert showed a peak intake in JulAug and considerably lower consumption throughout the year. This was also noted by Sharma (1994). Jackson (1980) observed a similar peak, however his deer ate Bramble throughout the year and it was the most important component of the diet in most months.

Ivy - Jackson (1980) saw an increase in Ivy consumption at the same time of year as Great Covert (JanFeb), however his deer took three times as much. Hosey (1974, 1981) and Sharma (1994) reported Ivy in the diet but in very small amounts and with no particular pattern over the year. Henry (1975, 1978) did not record any Ivy.

Reliability of the faecal analysis method for roe deer

The large proportion of Conifers in the diet at Great Covert was very similar to the study by Sharma (1994) but none of the others. Hosey (1974, 1981) found a similar pattern of intake over the year but not in the quantities that were witnessed here. This may also have been due to the numbers of Conifers that were brought down in the previous January storm (1990). Despite the collection of roe faecal samples commencing in September of that year, many of the fallen trees were still alive, not having had their root systems severely damaged. This may have also accounted for Sharma's (1994) data collected in 1989/1990. The importance of Bramble on the other hand, the mainstay of the deer at Squabb, was also found by Hosey (1974, 1981).

As for the fallow deer sites, all of the plant species found in the diet of roe deer have been noted by other researchers. Admittedly the quantities differ for some plant species but their presence in the diet coupled with similar seasonal patterns clearly indicates that the faecal analysis method of determining the diet was also reliable in the case of roe deer.

2.4.4.3 Individual plant species

When considering the volume of individual plant species in the diet, the majority were found to vary significantly at the two fallow sites and across the year, this was also true for the roe sites. On the basis of individual plant species (Section 2.3.3.2) there is clearly going to be seasonal variation in the amounts consumed over a year as plants have different stages of growth and senescence. At certain times of year plants contain more nutrients (Dietz *et al*, 1958; Dietz *et al*, 1962) or more secondary plant compounds (*e.g.* tannins, see Harborne, 1994) and may or may not be eaten on this basis. The large number of significant interactions for fallow and roe deer suggests that what the deer were eating may be much more closely linked to what was available rather than the nutrients or toxins the plants contain. It is assumed at this point that nutrient content of plant species peaked at the same time of year for all four sites as they were in a small geographical area where the geology was similar.

This interpretation of what the deer were actually doing is speculative at this point. Data from the availability measurements (Chapter 3) and chemical analyses (Chapter 4) will assist clarification.

2.4.5 Summary and general discussion

Determination of the diet of fallow and roe deer using faecal analysis has been shown to be a reliable technique. The types of plants eaten, and to some extent the amounts and seasonal variation, are comparable with published studies of fallow and roe deer diets. Therefore the volume data can be used unequivocally with the chemical information (Chapter 4) to investigate diet selection in fallow and roe deer (Chapter 5).

Seasonal variation of plants was highlighted in all three analyses, number of plant species eaten, occurrence of species in the diet and volume eaten. This was expected for the majority of plants, even evergreens, as they have periods emergence (in some cases), growth (when many are more palatable) followed by senescence. The analyses, number and occurrence of plant species in the diet both indicated that the two fallow sites, Brinken and Denny Lodge, were very different in what the deer ate and the amounts eaten over the year. The two roe sites, Great Covert and Squabb, were shown to be more similar.

Comparison with the work of other researchers (Sections 2.4.3.1 and 2.4.3.2) suggested that the actual amounts in the diet of fallow and roe deer are largely dependent on their habitat. This was also confirmed by the differences between Brinken and Denny Lodge for the same species of deer. These differences clearly indicate the necessity to take into consideration the availability of vegetation. These relationships will be investigated in more detail in Chapter 3.

CHAPTER 3 AVAILABILITY OF VEGETATION

ABSTRACT

The availability of all vegetation was estimated at four study sites: Brinken and Denny Lodge (fallow deer); Great Covert and Squabb (roe deer). Measurements were taken in mid-summer and mid-winter. The method used was adapted from published accounts to include plant availability in the vertical plane as well as the horizontal. Availability indices were compared to the number, occurrence and volume of plant species in the diet of the two species of deer at their respective sites. In general, these analyses suggested that in summer and winter there were no relationships between availability of plant species and their presence in the diet of fallow and roe deer. However, more detailed investigation of individual plant species did indicate that selection could be taking place, in summer more than winter and more by roe than fallow deer. Moreover, the data suggested that this selection of specific plants was not based upon availability alone but involved some other factor such as nutrient content.

3.1 INTRODUCTION

This chapter investigates the plant species that were available to the fallow and roe deer in their respective habitats and at different times of the year. The availability of vegetation is an extremely important measure. It could be the main influencing factor behind any diet selection theories put forward in this thesis as a result of the quantitative (Chapter 2 - Diet Composition) and the qualitative (Chapter 4 - Chemical Analysis of Vegetation) analyses that will be discussed in Chapter 5 (Diet Selection in the Natural Environment).

Most authors recognise the necessity of measuring the abundance or availability of vegetation when looking for reasons surrounding diet selection in animals (*e.g.* Weckerly and Kennedy, 1992). However there have been no published guidelines on the best methods of measurement for different animals. It is agreed by many to be a difficult task and appears to be dependent on the habitat being surveyed and the study animal. In the case of deer, the availability must be considered not only on the horizontal axis but also in the vertical (Tefler, 1974). A method for surveying vegetation was designed for this study incorporating methods used by previous workers (Bobek and Dzieciolowski, 1972; Perzanowski, 1990; Weckerly and Kennedy, 1992).

3.2 METHODOLOGY

In this section the study sites are referred to in Section 3.2.1 considering the ranges of both fallow and roe deer. The method used to measure the available vegetation is outlined in Section 3.2.2 and finally Section 3.2.3 details how the data were prepared and the statistical tests were employed.

3.2.1 Study sites

For detailed description of the study sites see Section 2.2.1.

Each study site (Brinken, Denny Lodge, Great Covert and Squabb) was too large to allow detailed investigation of vegetation availability so a section of each site was sampled (Table 3.1). This was an area known to be visited regularly by the deer through numerous sightings. The vegetation in these areas was thought to be representative of the whole site. Moreover, the sampled areas were considered representative of the deer's ranges, containing all types of vegetation and habitat.

In southern England (Dorset and Wiltshire) roe deer in coniferous woodland habitats (with some agricultural land) have been recorded as having ranges of between 7 and 22ha (Bramley, 1970; Johnson, 1984) with the males having only slightly larger ranges than the females (Johnson, 1984). Males are territorial from April to August, during this period their ranges are smaller than during the rest of the year (Bramley, 1970). The ranges of females overlap greatly with one another and also solitary males, they are occupied all year round but at parturition (mid May - mid June) are reduced in size (Johnson, 1984) and actively defended (Espmark, 1969). No significant differences have been found between ranges of the roe in summer and winter (Johnson, 1984), although Gent (1983) found some that individuals had smaller ranges in the winter. Johnson (1984) identified core areas within the ranges that were 44% of the total range. Using this figure and the known ranges of roe deer in the south of England a core area would be expected in the region of 3-10ha in size.

In contrast to the roe deer, fallow are non territorial and there is extensive overlap of their ranges.

Data on actual range size are scarce, but Putman (1986a) states that in the New Forest the mean summer range for females is 70ha (50-90ha) and 110ha (50-250ha) for the males, in winter these increase in size by approximately half. So, in contrast to the roe deer there are clear differences between the ranges of the two sexes. Core areas for fallow have only been recorded for males, as 35ha in the summer and 63ha in winter, approximately 33% and 41% of the entire range respectively.

Despite the seasonal ranges that occur for fallow and roe deer, each animal will have preferred areas within their ranges which they use on a daily basis in a relatively predictable manner (Putman, 1988). These were the areas chosen to assess the availability of vegetation in the current study.

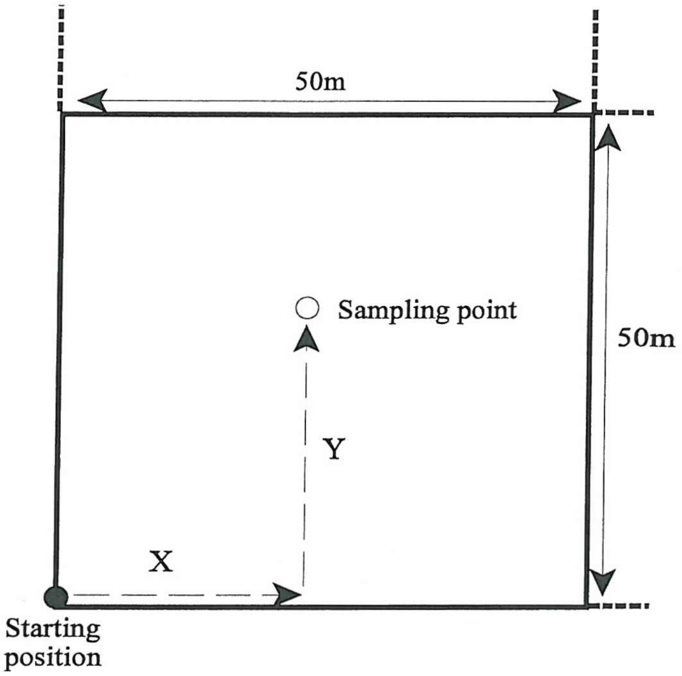
3.2.2 Measuring availability

Ideally the recording of available vegetation would have been done every bimonth enabling a direct comparison with the bimonth faecal sample data. However due to time constraints availability at each site was only measured twice; once in mid-winter (December-January 1996/7) and once in mid-summer (June-July 1996). This was thought to be sufficient since other researchers, when measuring vegetation supply for deer, assessed the availability twice a year at similar times (Perzanowski, 1990).

Each study site was divided up into 50m squares by aligning one side of a grid with one side of the site. A key feature was noted in the bottom left hand corner of each square (*e.g.* tree stump) if there were no natural markers a large stone or piece of wood was moved into place, this also became the starting point (Figure 3.1).

In every square a random number of uniform strides (between 1 and 50) were made across into each square and then another random number of strides were taken up into the square (Figure 3.1). At this point the species of vegetation was recorded at the browsing height for each species of deer, 1.2m above the ground for roe (Prior, 1993; Danilkin and Hewison, 1996) and 1.5m for fallow (Prior, 1993). If there were no plants at this height then an imaginary line was followed down to ground level, and the first example of vegetation within this column was recorded. It was important to measure the availability of vegetation in the vertical as well as the horizontal plane as both species of deer do not only eat at ground level. If there was no obvious vegetation within the column, the first piece of vegetation encountered immediately ahead of the experimenter was noted.

Figure 3.1 A 50m x 50m square within the grid covering each study site, showing how the random number of strides were made across (X) and up (Y) into the square, to reach the sampling point as indicated by the grey shaded circle.



In each square 12 plant species were recorded, Table 3.1 shows the total numbers of samples taken at each site during mid-summer and mid-winter. Unlike the faecal analysis all the samples were identified to species level, they could then be put into the relevant groups later for comparison with the faecal data.

Table 3.1 Numbers of plants sampled at each site in the summer and winter to calculate availability, and the total area sampled.

Site	No. of squares sampled	Total no. of plants sampled	Area over which samples were taken (ha)
Brinken	20	240	5
Denny Lodge	22	264	5.5
Great Covert	20	240	5
Squabb	21	252	5.25

The same grid system was used for the second sampling occasion, the natural or placed markers were still present. However, a different set of random numbers between 1 and 50 were used to generate the sampling points within each square.

3.2.3 Data analysis

The frequencies of each species of vegetation recorded were converted into proportions (Appendix 5) as different numbers of samples were taken at each site. Proportions allowed the data across sites to be compared.

3.2.3.1 Statistical analysis of the data

The availability data were statistically analysed using non-parametric tests requiring little or no knowledge of the distribution of the data. All hypotheses were tested using a critical probability (P) value of 0.05, unless otherwise stated. The following descriptions of each analysis is based on SPSS Base 7.0 for Windows, User's Guide (1996), Fowler *et al* (1998) and Dytham (1999).

Mann-Whitney U test: see Section 2.2.5.6

Wilcoxon Signed ranks test: This test is the non-parametric equivalent of the paired t-test, however here the medians of two matched pairs are compared. As for the paired t-test this test also looks at the difference between each matched pair and therefore the data must be interval measurements or frequencies. $P<0.05$ indicates that there is a significant difference between the median values of the two samples.

Spearman Rank correlation: This non-parametric test is designed to measure the relationship between two variables. The variables can be frequencies, proportions, indices or ordinal data as they are converted to ranks before the correlation coefficient (r_s) is calculated. $P<0.05$ indicates a significant correlation between the two variables measured, however r_s should preferably be 0.7 or more to signify a strong correlation despite the probability level. This is because r_s^2 gives the percentage of variation in one of the measures accounted for by the variation in the second measure ($r_s = 0.7$ gives 0.49 or 49%).

3.3 RESULTS

The first section considers the statistical analysis of just the availability data at each site (Section 3.3.1). The subsequent sections look at the relationships between availability and the three types of information gathered from the faecal analysis: number of plant species eaten (Section 3.3.2); occurrence of each plant species in the diet (Section 3.3.3); and volume or proportion of each plant species eaten (Section 3.3.4).

3.3.1 Availability of vegetation at each site

Plant species that were found to represent more than 1% of the total available vegetation in summer and winter are shown in Figures 3.2a-d. These data were also a measure of the diversity of species found at each site. The number of species present at each site in each season is the simplest index of diversity (Table 3.2).

Table 3.2 Numbers of different species available at each of the four sites.

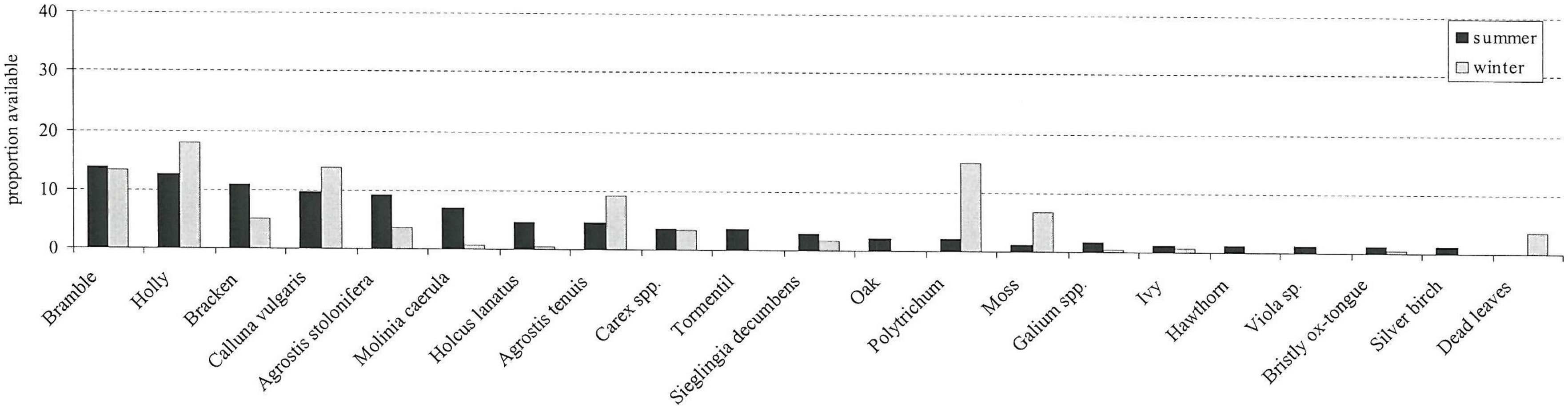
Site	Summer	Winter
Brinken	27	22
Denny Lodge	35	27
Great Covert	31	19
Squabb	30	20

These data suggest that there was little difference between each site and also between each season. These differences were tested using Wilcoxon Signed Ranks test to look at intra-site differences, *i.e.* seasonal differences. A Mann Whitney test examined differences between sites.

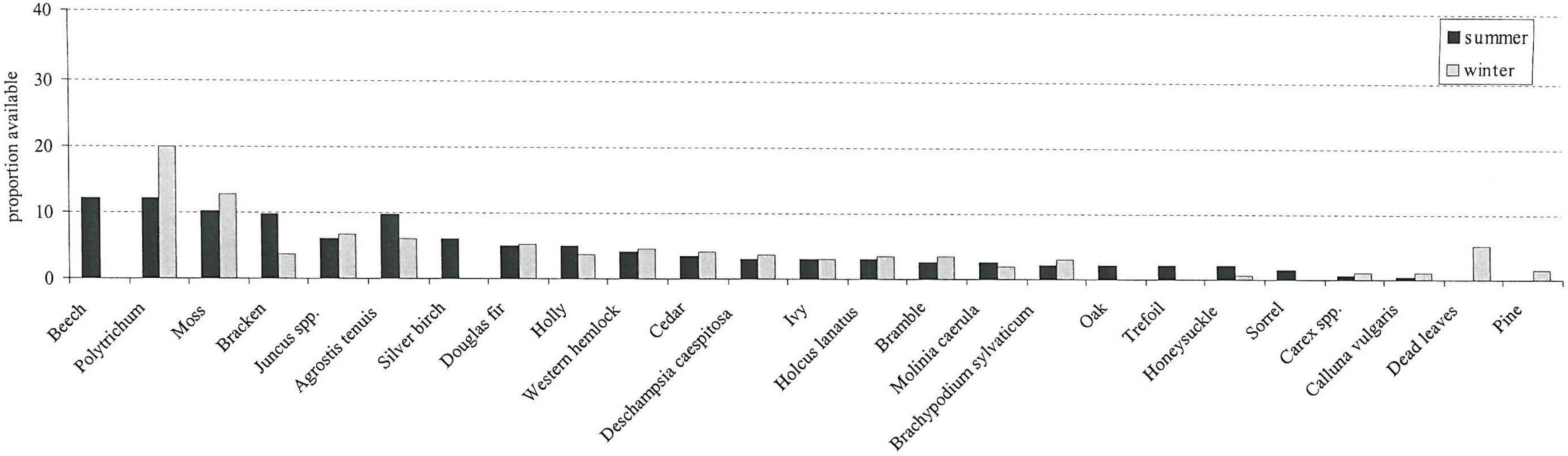
The Wilcoxon test was carried out on the two sets of data, summer and winter, for each species of vegetation. It was assumed that where a deciduous species of tree or a herb was found in the summer months but not in the winter the species was genuinely unavailable and therefore a zero was used for the winter value. In all other cases the species was classed as simply not being found, *i.e.* it

Figure 3.2a-d Availability of vegetation at all four sites in summer and winter

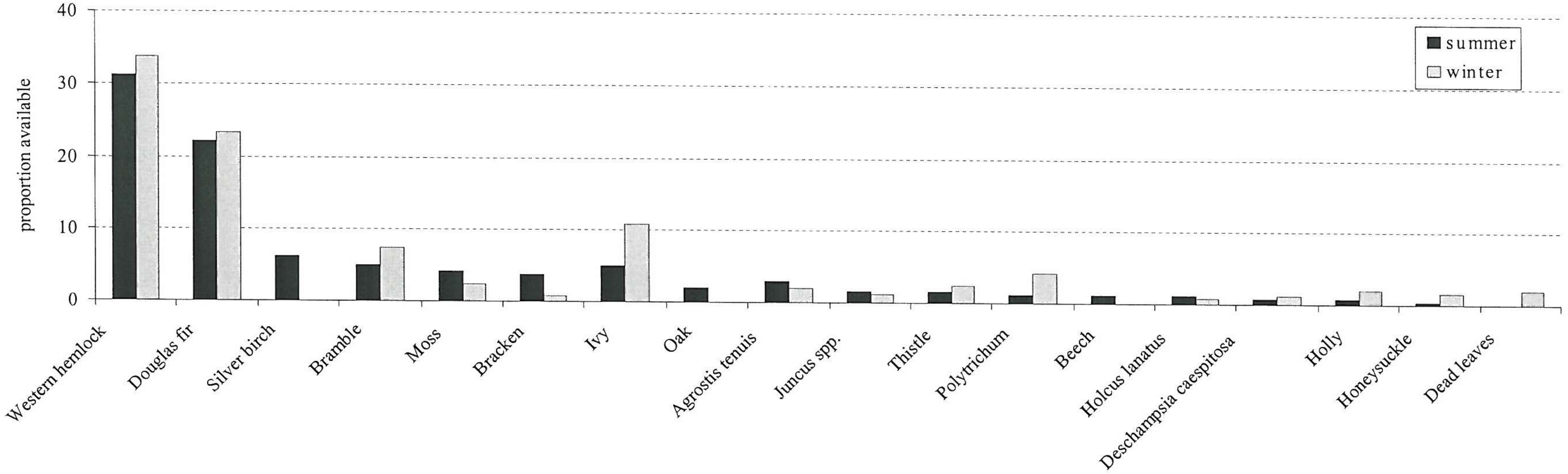
a) Brinken



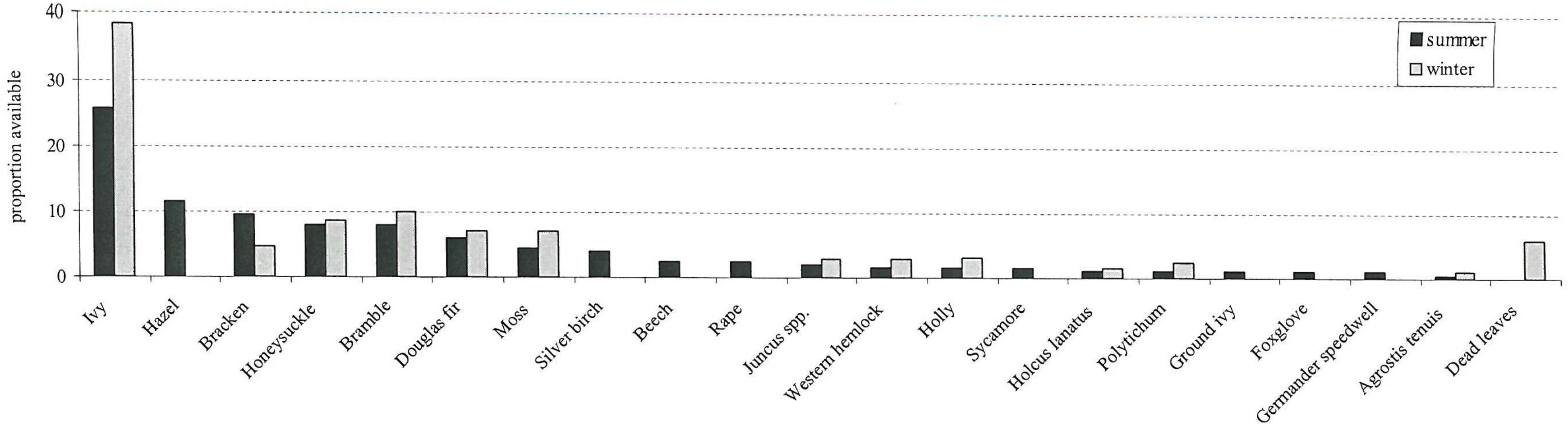
b) Denny Lodge



c) Great Covert



d) Squabb



was highly likely that it was present due to its presence in the other season, it was therefore categorised as missing. This was used for species found in the summer and not winter, and *vice versa*. This first analysis was used to test the following null hypothesis:-

> H_0 = no significant difference existed between the availability of vegetation during the summer and winter months within each site

The sample sizes were reduced as only species of vegetation that occurred in both seasons were used. The hypothesis was accepted as there was no significant difference between summer and winter availabilities at each of the four sites (Brinken: $Z = -0.854$, $N=25$, NS; Denny Lodge: $Z = -0.375$, $N=33$, NS; Great Covert: $Z = -0.373$, $N=25$, NS; Squabb: $Z = -0.229$, $N=30$, NS).

Differences between the two fallow sites and the two roe sites were also investigated for each season (summer and winter). Only data occurring at both sites being compared could be used therefore the sample sizes were reduced further (fallow: Brinken and Denny Lodge $N=20$; roe: Great Covert and Squabb $N=20$). The null hypothesis being tested here was as follows:-

> H_0 = no significant difference existed between the two fallow sites or the two roe sites for each season separately.

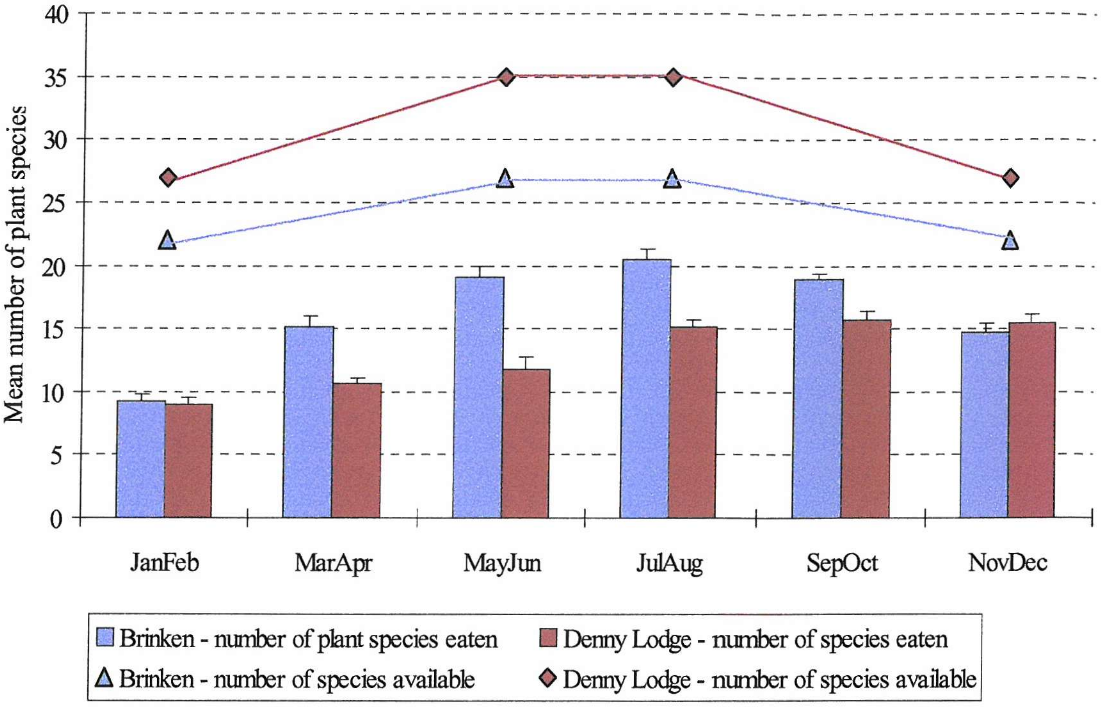
The Mann Whitney tests revealed no significant differences (fallow summer: $U = 160$, $N=20$, NS; fallow winter: $U = 174$, $N=20$, NS; roe summer: $U = 193$, $N=20$, NS; roe winter $U = 181$, $N=20$, NS), therefore the availability of the (common) plant species at the fallow sites and the roe sites were similar for each season.

3.3.2 Availability and Number of plant species eaten

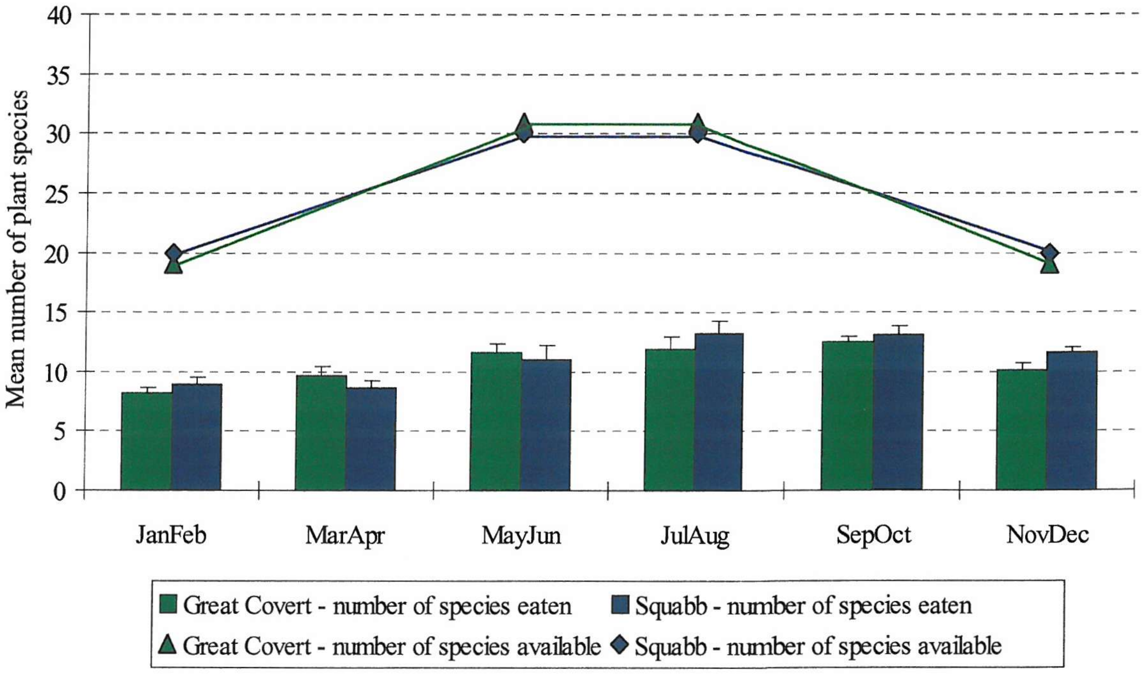
The number of plant species available was compared to the number of different plant species that the deer actually ate (Section 2.3.2). The relationship is shown in Figures 3.3a and b. As the availability measures were taken mid-summer (June/July) and mid-winter (December/January) the data were plotted for the four bimonths incorporated in the time scale, *i.e.* MayJun, JulAug NovDec and JanFeb. Statistical analysis of the data on the relationship between number of species eaten and availability was not possible as there were too few data points to make any test robust, therefore these results can only be descriptive.

Figures 3.3a-b Mean number of plant species (± standard error) eaten over the year at each of the four sites and the numbers of plant species available.

a) Fallow deer - Brinken and Denny Lodge



b) Roe deer - Great Covert and Squabb



The figures demonstrate that at all the sites the deer took far fewer numbers of species than were available to them. At Brinken (Figure 3.3a) the fallow deer took about three quarters of the number of species that were available to them in the early winter (NovDec) and less than half the number available in late winter (JanFeb). The numbers of species eaten in the summer months increased with the increase in number of species available, but still a third of those available were not eaten.

These differences were even more pronounced at Denny Lodge (Figure 3.3a). In the early winter they took only half the number of species available to them and in the late winter this had decreased to one third. The increase in availability in the summer months was not reflected by the same increase in numbers of species eaten, as it was for Brinken. Here, especially in early summer considerably fewer numbers (one third) of species were eaten. In late summer the numbers eaten did increase but still only to less than half the number available.

Figure 3.3a shows that, although not significant, the numbers of plant species available at Denny Lodge were greater than the numbers found at Brinken. It has already been shown that Denny Lodge was the site with the highest diversity of plant species in summer and winter (Table 3.2). However, this comparison with numbers of species eaten suggests that the deer eat only a proportion of the different species available to them. The reasons for this could be many-fold, *e.g.* digestibility, nutritional content, toxicity, physical attributes (thorns etc.).

Figure 3.3b shows that the two roe sites were extremely similar to each other in the numbers of plant species that they had to offer in the summer and the winter. However, the availability far exceeded the actual numbers of species eaten for the roe deer at Great Covert and at Squabb. The deer at both of these sites took similar numbers of species throughout the year (see Section 2.3.2, multiple range test indicated no significant difference between the roe sites). In the winter months they took approximately half the numbers of species available. In the summer, although the numbers available increased dramatically the numbers eaten did not follow suit, and the deer ate just over a third of what was available.

These differences between numbers available and numbers eaten for the roe deer compared to the fallow deer may be indicative of selective feeding by roe deer more so than fallow deer. The latter are known to eat more grasses, particularly in the spring and summer (Jackson, 1974, 1977; Putman *et al*, 1993). The current data showed that at Brinken and Denny Lodge they ate up to 2½ times as many grasses in the summer than the roe deer (Appendix 5). This is a possible explanation for the difference in the numbers of species eaten between fallow and roe deer in the summer months.

3.3.3 Availability and Occurrence of species

The occurrence data measured the presence and absence of particular plant species in the diet, *e.g.* Pine occurred in 15 of the 20 samples in JanFeb at Brinken (Section 2.3.3, Appendix 3). In this format (*i.e.* 15 samples) it could not be directly related to the availability information and was therefore converted into proportions of the total number of samples, thus the value for Pine would be 75%. The faecal data were originally collected for each month separately and so for a more informative comparison between availability and occurrence of plant species, the occurrence data were recalculated for summer (July and August) and winter (December and January).

The sample sizes were governed by those plant species that had measures for availability and occurrence in the diet, hence they were not identical for summer and winter at each site (Brinken: summer N=17, winter N=17; Denny Lodge: summer N=23, winter N=22; Great Covert: summer, N=18 winter N=13; Squabb: summer N=17, winter N=13). Spearman Rank Correlation was used to investigate whether there was any relationship between the two measures. The null hypothesis tested was as follows:-

> H_0 = no significant relationship existed between the availability of plant species and their occurrence in the diet.

This was tested for each season and at each site. For the fallow sites, Brinken and Denny Lodge, there was no correlation between availability and the occurrence of plants in the diet in either summer or winter (Brinken: summer $r_s = -0.036$, NS; winter $r_s = 0.375$, NS; Denny Lodge: summer $r_s = -0.08$, NS; winter $r_s = 0.268$, NS). However, at Great Covert there was a strong significant positive association ($r_s = 0.633$, $P<0.05$) between availability and occurrence of the same species in the diet during the winter. This relationship was not evident in the summer ($r_s = 0.416$, NS) nor at Squabb in either summer or winter ($r_s = 0.209$, NS, $r_s = 0.369$, NS respectively). The results suggest that the availability of plant species or groups has little impact on the diet of both species of deer, except for roe deer at Great Covert in the winter.

3.3.4 Availability and Proportion of species eaten

The proportion of the diet made up of each plant species was compared to its availability to see if there was any relationship between how much of a particular species they were eating and how available it was in the surroundings. The plant species that were used are shown in Appendix 5; only those that comprised ≥1% of the diet in any one bimonth and whose availability was recorded. As the availability was measured in June/July and December/January, the proportion of each species listed in Appendix 5 were recalculated from the original data which was collected for every month and the values listed are for JunJul and DecJan bimonths.

Again the sample sizes were not identical between summer and winter for each site as plant species that were present in one season and not in the other (except for deciduous trees or herbs) were classed as missing (Brinken: summer N=16, winter N=15; Denny Lodge: summer N=16, winter N=15; Great Covert: summer N=11, winter N=10; Squabb: summer N=14, winter N=12).

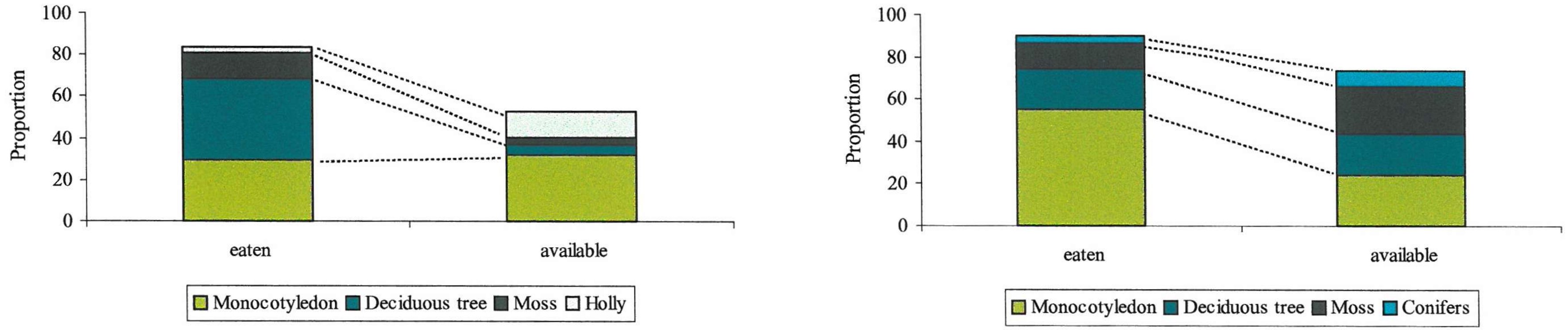
As with the occurrence data, Spearman Rank Correlation was used to investigate whether there was any relationship between the two measures. The null hypothesis was as follows:-

> H_0 = no significant relationship existed between the availability of plant species and proportion of the diet they constitute.

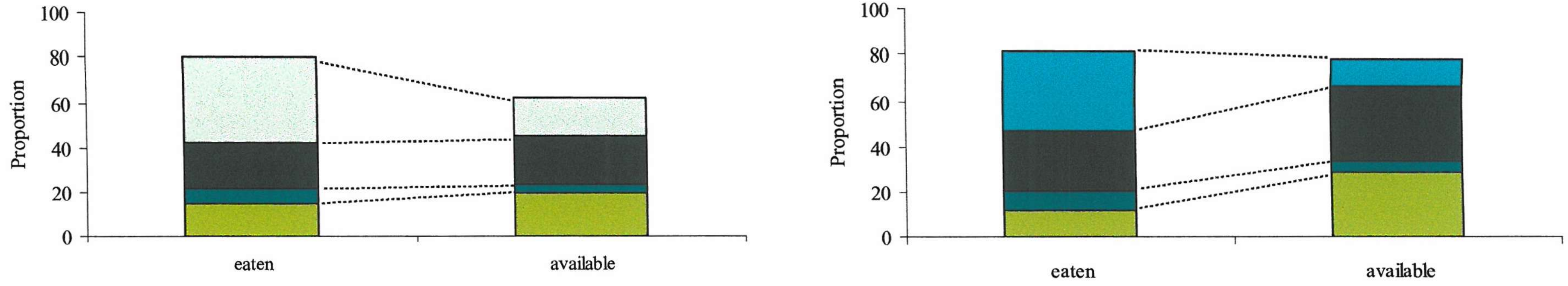
Correlations for the summer availability of plant species and their proportion in the diet were not significant for any of the sites (Brinken: $r_s = 0.378$, NS; Denny Lodge: $r_s = -0.312$, NS; Great Covert: $r_s = 0.183$, NS; Squabb: $r_s = 0.115$, NS). Therefore the amounts of each species the deer ate were not significantly influenced by their availability in the immediate environment. This was also the case in the winter season. Availability did not significantly affect the amounts of plant species eaten at both fallow sites, (Brinken: $r_s = 0.329$, NS; Denny Lodge: $r_s = 0.29$, NS) nor at both roe sites (Great Covert: $r_s = 0.522$, NS; Squabb: $r_s = 0.299$, NS). These results suggest that overall the availability of plant species in the local environment had no effect on how the diets of the deer were made up. The plant species forming the larger sections of the diet were not those that were most available in either summer or winter.

The relationship between the proportions of plant species found in the diet compared to their availability in the environment was investigated further by examining the plant species eaten in the highest quantities by fallow and roe deer at the respective sites (Figures 3.4a-h).

Figures 3.4a-h Proportions in which the most common plants at each site were available and were eaten in summer and winter.

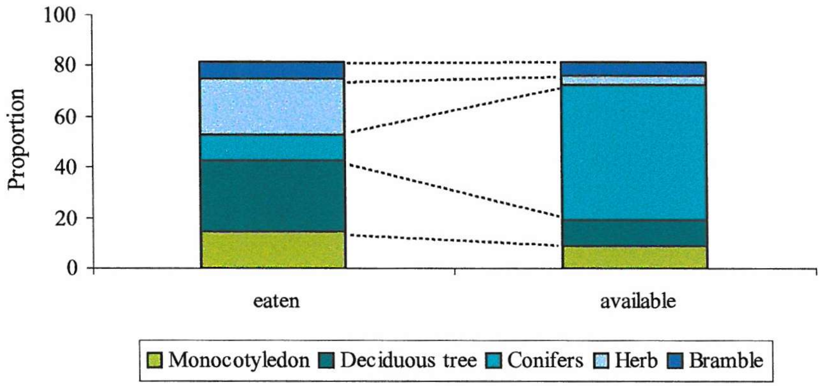

a) Brinken - summer

c) Denny Lodge - summer

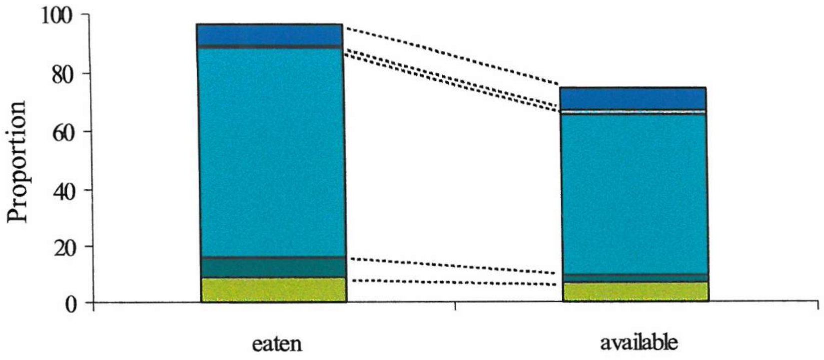

b) Brinken - winter

d) Denny Lodge - winter

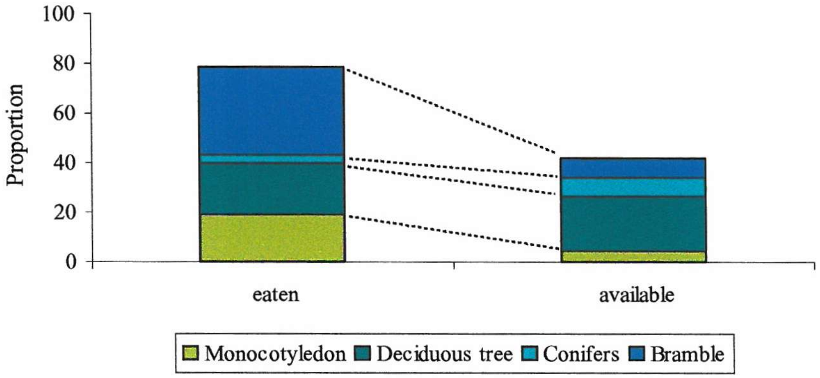
e) Great Covert - summer



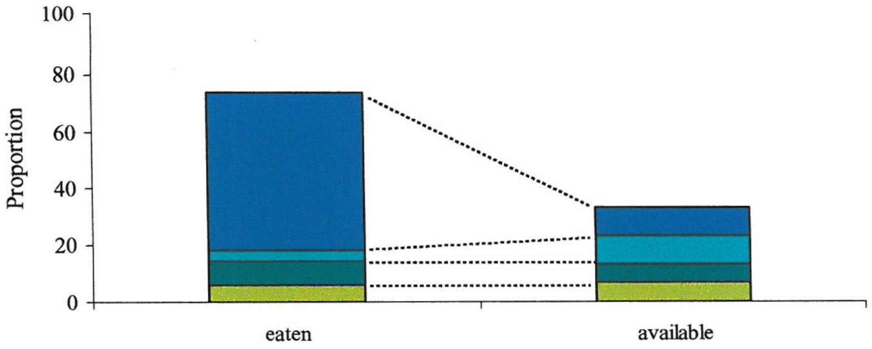
f) Great Covert - winter



g) Squabb - summer



h) Squabb - winter



The data from Figures 3.4a-h have been summarised in Table 3.3 indicating where the proportions eaten were greater than, less than or approximately equal to the proportions available in the habitat. The shaded cells show the contribution to the diet by these particular plant species or groups which was at least twice as great or small as their availability in the diet would have predicted.

There are two possible explanations for these findings. The deer may have been foraging outside the study sites. This was quite possible for the fallow deer where the summer ranges for females is 70ha (50-90ha) and 110ha (50-250ha) for males, and in winter these increase in size by approximately half (Putman, 1986a). The sizes of Brinken and Denny Lodge sampled (overall, not just for availability) were 35 and 55ha respectively. However, for the roe sites this was less likely to be the case as the published ranges for roe deer in the south of England are 7-22ha (Bramley, 1970; Johnson, 1984) and the roe sites, Great Covert and Squabb were 75 and 70ha respectively, making them more than adequate to sustain several overlapping roe deer ranges.

Another suggestion was that the deer were actively selecting for or against specific plants, so for example, although the availability may be low they took a lot of a particular species making it an important component of the diet. Assuming that plants are eaten in the same proportions as their availability, more selection took place in the summer; on eight occasions there was selection for certain plants (Table 3.3) and there was no notable difference between the fallow and roe deer (fallow three occasions; roe five occasions); on only three occasions was there selection against specific plants (Table 3.3). A trend was evident within the species or groups of vegetation that were selected for, Monocotyledon was clearly selected for at three of the four sites, only at Brinken was this not apparent; Deciduous tree was selected for at Brinken and Great Covert, and was approximately equal in its availability and consumption at the other two sites. At Brinken, Moss was selected for but not at Denny Lodge. Bramble was selected for at Squabb whereas at Great Covert the proportion available was approximately the same as that eaten. Herbs were much more abundant in the diet at Great Covert than their occurrence in the environment would have predicted and therefore appear to have been selected for. Selection against certain plants was apparent at Brinken where more Holly was available than was eaten, at Great Covert and Squabb more Conifers were available than eaten, particularly at Great Covert.

In winter the number of occasions plants were selected for was reduced to four, two of which were the same as in the summer (Great Covert: Deciduous tree; Squabb: Bramble), the other two were Holly at Brinken and Conifers at Denny Lodge both of which were in sharp contrast to the summer results particularly for Holly which was selected against in the summer. There were seven occasions

Table 3.3 Relationship between the proportions of specific plants available and the proportions found in the diet, at the different sites in summer and winter

Legend: e = proportion eaten; av = proportion available; ≈ = approximately equal to; light shaded cells indicate where positive selection (selection for) may have been occurring (*i.e.* proportion eaten is greater than proportion available); darker shaded cells indicate where negative selection (selection against) may have been occurring (*i.e.* proportion eaten is less than proportion available) .

Site	**Species eaten in highest quantities**	**summer**	**winter**
Brinken	Monocotyledon	e ≈ av	e < av
	Deciduous tree	e > av	e > av
	Moss	e > av	e ≈ av
	Holly	e < av	e > av
Denny Lodge	Monocotyledon	e > av	e < av
	Deciduous tree	e ≈ av	e ≈ av
	Moss	e < av	e < av
	Conifer	e < av	e > av
Great Covert	Monocotyledon	e > av	e ≈ av
	Deciduous tree	e > av	e > av
	Conifer	e < av	e > av
	Herb	e > av	e ≈ av
	Bramble	e ≈ av	e ≈ av
Squabb	Monocotyledon	e > av	e ≈ av
	Deciduous tree	e ≈ av	e ≈ av
	Conifer	e < av	e < av
	Bramble	e > av	e > av

where the proportion of available plant species was approximately the same as the proportion actually eaten, three of these were the same as in the summer (Denny Lodge: Deciduous tree; Great Covert: Bramble; Squabb: Deciduous tree). On two occasions plants were eaten in higher proportions than they were available but not twice the amount, they were therefore not considered as being selected for, however they may still be important, these were Deciduous trees at Brinken and

Conifers at Great Covert. The remaining plants were those that were available in higher proportions than they were eaten (selection against): Monocotyledon at Denny Lodge and Conifers at Squabb, the former was selected for in the summer whereas the latter was also selected against in the summer.

3.4 DISCUSSION

More plant species were available over the summer months than the winter at all four sites. This is due to the life cycles of most plants, whether perennial, biennial or annual they all produce new growth in the spring. Of the plants available in the summer many were also available in the winter, hence the non significant result in the Wilcoxon test for the fallow and roe sites. However there were plant species only available at specific sites and not others (Appendix 5). The availability of food can vary greatly from one location to another, so comparisons between the actual species eaten in the diets of any animal can be of limited use. This has been found by researchers working on the composition of deer diets, particularly roe deer (Jackson, 1980; Holisova *et al*, 1986; Tixier and Duncan, 1996; Cornelis *et al*, 1999). These authors state that it is the habitat, and not the seasons, that is responsible for the availability of foods moreover that major differences in food availability are still possible in the same type of habitat.

Using plant availability Weckerly and Kennedy (1992) considered two hypotheses for explaining the feeding strategies of white-tailed deer. They examined the forage abundance hypothesis (FAH), which originates from optimal foraging theory (see Stephens and Krebs, 1986) and the selective quality hypothesis (SQH). The former considers availability as the important variable that influences diet selection and the latter argues that it is plant quality.

According to the FAH when food sources increase, animals become more selective to optimise a nutrient or mixture of nutrients because of increased foraging efficiency. When food sources decrease animals become less selective and are influenced more by availability. This model assumes that most of the available foods are palatable to deer (Weckerly and Kennedy, 1992).

In contrast the SQH argues that at times of high plant availability animals are less selective because high quality food is in greater supply and selectivity is higher during times of low availability. This model assumes that only a small proportion of the total food available is palatable (Weckerly and Kennedy, 1992). The current results are now discussed in light of Weckerly and Kennedy's findings

(1992) and their support for the FAH and SQH evaluated.

At all of the sites there was an increase in availability and the number of plant species taken in the summer, this could indicate that what was eaten in this season was influenced by availability. However the roe deer took far fewer species than were available especially in the summer. This suggests that they are showing selection but not based on availability. It could indicate that many of the plant species contain toxins or are low in digestibility. This last suggestion is less likely as fibre increases in autumn and winter rendering the food less digestible (Putman *et al*, 1981), in the summer this should not have been the case. Toxins especially tannins build up as the growing season progresses (Harborne, 1994). They would be expected to peak in autumn, so this is not a likely reason either. Therefore some degree of selectivity particularly in the summer appears probable. According to the FAH when food is abundant (as it appeared to be in the current study with high numbers of species available) deer are more selective and availability is not the major influencing factor. The findings from this part of the study suggest the FAH was operating here.

The occurrence of species in the diet when compared to availability showed no relationship existed at the fallow sites, Brinken and Denny Lodge nor at Squabb (roe site) in either season. At Great Covert roe appeared to show some selection based on availability in the winter, indicated by strong positive correlations, but this disappeared in the summer. However, it must be taken into consideration that occurrence in the diet did not reflect the amount the deer were eating of a particular plant species, it simply measured presence or absence. Therefore the relationships that existed did not necessarily mean that if a species was readily available the deer would eat a lot of it. So here, if a species is highly available then it will be present in the diet of several deer at that particular time, but not necessarily in large amounts. The results from this analysis suggest that overall availability had very little effect on what the deer ate, except for roe deer at Great Covert in winter.

A more reliable measure to look at selectivity in terms of availability is the proportion or volume of each plant species in the diet as used by Weckerly and Kennedy (1992). At all the sites for each season the relationship between proportion represented in the diet and the environment was not significant, the quantities of plants in the diet were not influenced by availability.

Further examination of the proportion of individual plant species showed that some species figured more prominently in the diet than their presence in the environment would predict. The deer could have been either foraging further afield, though still within their ranges or they could have been showing selection. The former explanation was less likely for the roe (Section 3.3.4) and so this leaves selection as a plausible interpretation. The overall pattern that emerged appeared to suggest that certain plants were selected for and against in the summer, others in the winter and others throughout the year. However, there was more selection occurring in the summer months than the winter at each site and roe showed more than fallow.

3.4.1 Summary and general discussion

In general there appears to be no selection of diet based upon the availability of vegetation in the environment. Weckerly and Kennedy's findings (1992) rejected the FAH as availability did not have a great influence on diet composition. Their data supported the SQH, when forage was abundant white-tailed deer were less selective. Weckerly and Kennedy (1992) found high positive correlations between amounts eaten and availability in summer, unlike the current study which found no correlations in the summer. In accordance with the present study they found no correlations in winter when availability was low and dietary fibre high, suggesting selectivity for certain foods was not based on availability but more likely on quality. The examination of individual plant species and groups suggested that selection occurred in the summer as well as the winter and diet selection was not based upon availability. This part of the current study cannot accept the SQH, in fact it shows more support for the FAH. Without further analysis a definite conclusion can not be drawn. This will be addressed in the following Chapter by investigating the quality of the diet eaten by fallow and roe deer. This, in turn, will be combined with the quantitative data to look specifically at diet selection in the natural environment (Chapter 5).

CHAPTER 4 CHEMICAL ANALYSIS OF VEGETATION

ABSTRACT

The most common plant species in the diets of fallow and roe deer were chemically analysed for their nutrient content (nitrogen, calcium, magnesium, potassium and phosphorus). Plants were collected from four study sites (Brinken, Denny Lodge, Great Covert and Squabb) at the same time as faecal samples, thus producing an annual nutrient profile for each plant species. Seasonal variation in nutrient content was clearly evident, with plants showing increased concentrations in spring/summer, except for calcium which peaked in winter. Greatest seasonal variations were noted in plants that had distinct annual growth cycles, *e.g.* deciduous trees, and were lowest in coniferous plant species. Overall mean nutrient contents varied across the plant species, in general deciduous plants contained higher proportions of most nutrients than evergreen species, except for calcium. Differences were found in the overall plant nutrient content at each site, with Brinken (fallow deer) being less rich in nutrients than the other sites, possibly due to the acidic nature of the soil.

4.1 INTRODUCTION

To understand the biological basis for diet selection, the quality of the diet has to be determined in some form. The aim of this chapter is to answer the question **"do the different forage plants vary in their nutrient content over the year?"** This chapter will attempt to answer this question by looking at the nutrient content of the plants at each site.

The quality of the plant samples collected at each site over one year was investigated by chemically analysing their macronutrient content. Macronutrients are those nutrients (chemical elements) required in large quantities by plants and animals, as opposed to micronutrients which are required in smaller amounts. The macronutrients investigated here were nitrogen, calcium, magnesium, potassium and phosphorus. All of these nutrients are assimilated into plants through their root systems. They are absorbed as ions from the soil solution. The concentration of these ions largely depends on soil chemistry. The relationship between soil chemistry and plant chemical content is very complex and relies on many factors including the geology of an area and the prevailing weather conditions (see van Soest, 1994).

Once the nutrients have been absorbed by plants, animals ingest the plants, thereby obtaining the macronutrients (and micronutrients) they require. In most cases their requirements are met by eating several different plants. Within both plants and animals these macronutrients have important functions, the details of which can be found in Appendix 6. Macronutrients will be called *nutrients* for the remainder of this thesis for simplicity.

Measures of specific nutrients in plants can be found in the literature together with methodologies for their analysis (Allen, 1989; Jones *et al*, 1991). However, there have been very few published accounts of the nutrient composition of plants eaten by deer. Of these most have only investigated the nutrient content in one season, usually winter as this is when most nutrients are thought to be limiting to deer (mule deer: Smith, 1957; white-tailed deer: Hellmers, 1940; Torgerson and Pfander 1971; black-tailed deer: Radwan and Couch, 1974) or at one specific point in time (white tailed deer: Swift, 1948). One of the most relevant and comprehensive descriptions of the nutrient contents of plants was carried out by Dietz *et al* (1958, 1962). They looked at the principal browse species in the diet of mule deer. However, this detailed analysis was conducted in an area of Colorado, USA, where the terrain and climate are very different to that of southern England. Similar work carried out in the locality of this current research is limited to Ekins (1989). She investigated the forage resources of cattle and ponies in the New Forest including qualitative analysis of vegetative samples. Although this work did not look specifically at the diet of deer in the New Forest, it did include nutrient analysis of plant species commonly found in the diet of the deer in this study. Some work has looked specifically at the nutrient content of plant species eaten by fallow deer in the New Forest, but unfortunately it has not been published and is only referred to by Putman (1986a).

Much of the work on the nutrient composition of specific plants was carried out between the 1950s and '70s. The techniques used were however the same as many used today, although less automated, so the results are thought to be reliable. As the nutrient composition of plants is very dependent on the prevailing soil chemistry, which varies with location, direct comparisons with published research should be limited (with the exception of Ekins [1989] work), but investigating similarities in seasonal patterns may nonetheless be informative.

In this study nitrogen was chosen as it gives a measure of the amount of protein present in each species of forage (crude protein = N x 6.25; see Appendix 6). Protein is important as it has been suggested as a limiting factor for deer particularly in winter (Parfitt in Putman, 1986a). The other nutrients measured all have vital roles in body function (Appendix 6). All have been analysed by other researchers working with herbivores (*e.g.* Dietz *et al*, 1958, 1962; Short *et al*, 1966; Ekins, 1989) and their requirements by deer have been investigated (*e.g.* French *et al*, 1956; Ullrey *et al*, 1967). It has been suggested that some of these nutrients are specifically selected for by some deer (*e.g.* phosphorus: Verme and Ullrey, 1984; Schultz and Johnson, 1992).

This chapter investigates the nutrient content of plant species that were important components of fallow and roe deer diets. Primarily, these data will be used together with the faecal analysis results (Chapter 2) to look at diet selection in these two species of deer (Chapter 5). However, the nutrient contents of plants are of great interest in their own right as they provide valuable information on what different species of plants contain, their seasonal variation and differences that may or may not occur between sites in the same localities. The trends observed and hypotheses generated from the analyses may also help in the interpretation of any diet selection that may be suggested in Chapter 5.

4.2 METHODOLOGY

Chemical analyses were used to investigate the nutritional content of fallow and roe deer diet. A profile of the diet at each site was made up from the quantitative results of the faecal analysis (Chapter 2), this showed what species of vegetation the deer were eating and also in what quantities. Using this information samples of vegetation that occurred in at least 1% of the diet in any one bimonth were chemically analysed (Appendix 7). Nitrogen, calcium, magnesium, potassium and phosphorus contents were measured.

4.2.1 Study sites

In order to gather information on the nutrient profiles of vegetation actually eaten by the same fallow and roe deer as supplied the quantitative data via faecal analysis, samples of plant material were collected from the same study sites. Descriptions and locations of the sites are in Section 2.2.1.
The model used in the analyses of the data was the same as for the faecal analysis, model: *response = site + bimonth + site*bimonth.* In this case differences between all sites were investigated as each was potentially distinctive in the nutrient content of its vegetation.

4.2.2 Collection of the samples

On the same monthly visit as when the faecal samples were collected, samples of all available vegetation were taken. This was done in the form of 'pluck samples'. The vegetation was selected using several criteria. Vegetation was not taken from above the height the respective deer could reach (1.2 m above the ground for roe [Prior, 1993; Danilkin and Hewison, 1996] and 1.5 m for fallow [Prior, 1993]); newer growth was selected where there was the choice (see Provenza and Balph, 1990); several leaves were taken from different plants (where possible) to represent one species; whole leaves were picked and flowers were taken in conjunction with leaves when present. For each site, samples were taken at approximately the same time of day each month (± 1 hour) between the hours of 10am and 3pm, although diurnal changes in nutrient content are thought to be relatively small (Allen, 1989). All samples were unbroken whole leaves/flowers and were placed together in a plastic bag. Within one hour of collection the vegetation was taken back to the laboratory, identified and placed into labelled paper bags with all the individual species kept separately.

4.2.3 Preparation of the samples

The vegetation samples were air dried in the paper bags (see Dietz *et al*, 1958) at room temperature in the same way as the faecal samples (Section 2.2.3). It was important not to place the samples straight into the paper bags if they were wet, *i.e.* from rain or dew, as microbial decomposition could ensue also, if drying is prolonged, metabolic changes may take place (Allen, 1989). In cases where samples were wet they were air dried at room temperature for periods of up to three hours on a metal tray. As with the faecal samples, the bags were checked for signs of microbial contamination and breakdown. On finding any signs of such activity the samples were immediately discarded, very few samples were lost in this way.

In order to obtain a representative sample, the dried vegetation was milled using a Gallenkamp mill (a set of knife blades that rotate in opposition to another set fixed around the edges of the grinding chamber) and passed through a 0.5mm mesh sieve (see Allen, 1989). This allowed each sample to be thoroughly mixed enabling the whole leaf to be represented. The samples were then dried in an oven at 80°C to remove any last traces of moisture, cooled and stored in labelled, sealed glass vials in a cool, dark place.

For the nitrogen analysis these dried samples could be used without any further preparation. Analysis of the other nutrients required some additional preparation. The samples underwent acid digestion, also known as a wet digestion, where oxidising reagents breakdown the organic matter. There are several different kinds of acid digestion using different acids or mixtures of acids. Nitric acid (HNO_3) digestion was chosen in this case as it was a relatively simple and less hazardous than many of the others. This method has been used successfully for sediments (*e.g.* Krumgalz and Fainshtein, 1989) and plant matter (*e.g.* Zarcinas *et al*, 1987).

Acid digestion was carried out in 13ml screw top polypropylene tubes. These were labelled and approximately 200mg of dried vegetation (range 100-250mg depending on amount of sample collected) were weighed out, the exact weight was recorded. The weighed samples were put into the labelled plastic tubes and 2ml of HNO_3 added. The lids were screwed on loosely to allow any pressure to escape and placed into a heating block in a fume cupboard. They were heated at 80°C overnight (12-20 hours), after which time all the solid matter had been digested. When the samples were cool 10ml of deionised water were added to each tube, shaken and sealed. The samples were then stable and could be stored in a fume cupboard and for up to six months (K. Collins pers. comm.).

4.2.4 Nitrogen analysis

Nitrogen analysis was carried out using a Carlo Erba Instrument CHNS-O EA 1108 Elemental Analyser. This method used very small amounts of sample (1.3-1.6mg) encased in a tin capsule, which was placed in a chamber and heated, melting the tin. Each sample went through a series of oxidation and reduction reactions and a calorimeter measured the thermal output, this was converted to millivolts. The computer, which had been calibrated to measure nitrogen, calculated the content in the sample. The machine was calibrated at the start of each session using blanks and a standard (acetanilide). The standard was run between every five test samples to check the machine was working correctly and also to provide a correction factor for each sample. On the advice of the technician (S. Akbari) it was not necessary to do any replication for a number of reasons: each sample was homogeneous, the amount used was very small and the machine was checked regularly. After each session the output from each sample was corrected using the correction factor from the acetanilide standard, and the percentage of nitrogen in each sample was recorded.

4.2.5 Calcium, magnesium and potassium analysis

To analyse the calcium, magnesium and potassium content a Pye Unicam SP9 atomic absorption spectrophotometer was used. This machine was set up specifically for each element, a lamp emits light at the correct wavelength for the element and the sample was drawn in causing an oxy-acetylene flame to burn a certain colour. This colour was measured within the specific wavelength for each element, converted to millivolts and recorded as a peak on a chart recorder. Standards (commercially available) and blanks were made for each element and run every 25 samples (as recommended by the usual operator, K. Collins). The vegetation samples were run for each element and were put through the machine twice to provide a mean peak height. In the case of magnesium and potassium the samples had to be diluted by a factor of 100 in order to get a measurable reading. Using the calibration from the standards, the mass of sample used and the volume of solution, the percentage of each element was calculated to give percentage dry matter (% dry weight) of nutrient.

4.2.6 Phosphorus analysis

Phosphorus was analysed using a Hitachi U-2000 spectrophotometer. For this analysis the machine was set up to read at 882 nanometers (specific for phosphorus) to measure the absorbance of the samples which could then be converted to percentage of phosphorus. The following reagent was made up every time the machine was used, 20ml ammonium molybdate, 50ml sulphuric acid, 20ml ascorbic acid and 10ml potassium antimonyl tartarate. A stock standard phosphate solution was made up by dissolving 0.816g of (anhydrous) potassium dihydrogen phosphate in a 1 litre volumetric flask with MQ (phosphate free) water, this gave a solution containing 6 millimoles of phosphorus per litre. From this standard phosphate solutions were made, for this analysis these were 3, 9, 15 and 24 μmoles phosphate per litre (*e.g.* to make a 15 μmoles standard means diluting the stock standard solution 400 fold). Reagent was added to each of the standards, the blanks (MQ water) and also to the samples (0.5ml reagent to 5ml of standard, blank or sample). These were then left for 10 minutes to turn blue. The spectrophotometer was set to zero using MQ water with no reagent and then calibrated using the standards, the samples were then run through the machine. The absorbance readings from the standards were used to produce a calibration curve from which the phosphorus content of each sample could be derived. Standards and blanks were run every 50 samples (on the advice of the technician, P. Gooddy) each sample was tested three times and a mean taken.

4.2.7 Digestibility and energy content

For a complete profile the digestibility and energy content of each vegetation sample was required, unfortunately, in most cases insufficient plant material had been collected from each study site. Digestibility estimation alone requires 2g of sample. Energy values do not differ greatly over the year (R.J. Putman pers. comm.), however it was thought necessary to have some values of digestibility, so other authors' values were used. Previous studies have been done in the New Forest (Putman *et al*, 1981; Ekins, 1989) and these values for digestibility will be used later. Differences in the digestibility of plant species at these sites and those in the current study, were believed to be minimal due to the similarity in sites. The methods for obtaining these values are outlined in Ekins (1989) with the method for determining digestibility taken from Jones and Hayward (1975). The digestibility values will be used sparingly and will only be incorporated into the analysis considering important forage species in the diet which will in turn be used to consider diet selection (Chapter 5).

4.2.8 Data analysis

4.2.8.1 Trends in quality of vegetation at each site

Much of the chemical analysis data were combined to make composite groups (Section 4.3.3), in particular the herbs and grasses. The reason for this was that some plant fragments from the faecal analysis could not be identified down to species level, only to group (Appendix 2), and as the quality data would ultimately be coupled with the diet composition data to investigate diet selection (Chapter 5) these had to be measured in the same groups. As this was the case and in order to use all the data before they were consolidated, nutrient profiles of the vegetation at each site were constructed. Each site will be considered separately for each nutrient (Section 4.3.4).

This analysis will reveal general seasonal trends for each nutrient, these can then be compared to the published literature in order to check the reliability of the experimental techniques used to measure nutrient content in the same way as for the faecal analysis (Section 2.4.4.1)

4.2.8.2 Trends in quality of important forage species

This analysis looked at the trends in diet quality in much more detail. Different species of plants occurred at the four different sites and as many as possible of these were chemically analysed. In order to look specifically at variation in nutrient content between species (interspecific), across bimonths (seasonal variation) and between sites (inter-site), only species common to **all** four sites and which formed a major part in the diet of the deer at each site were compared. All plant species (but not groups) that represented ≥5% of the diet (Appendix 3) at any one site were compared (Section 4.3.5). These data will provide a comparison with Ekins' (1989) data which will also assess the reliability of the techniques used. Published digestibility values could only be considered for the important plant species as these were the only ones that were analysed by Putman *et al* (1981)[1].

The analyses described in Sections 4.2.8.1 and 4.2.8.2 may appear similar, however they evaluated very different aspects of nutrient content. The first used all the vegetation data to look at seasonal trends irrespective of how much of each was eaten in order to make comparisons with other studies and test the methodology. The second looked specifically at those species of plants important to at least one species of deer at one site, which will help with the interpretation of diet selection. It also allowed the methodology to be tested but more directly with a study carried out in the same locality (Ekins, 1989)

4.2.8.3 Statistical analysis of the data

Non-parametric and parametric tests were used to test the data (see Section 2.2.5.6 for assumptions and data requirements). All hypotheses were tested using a critical probability (P) value of 0.05, unless otherwise stated. The following descriptions of each analysis is based on SPSS Base 7.0 for Windows, User's Guide (1996), Fowler *et al* (1998) and Dytham (1999).

Levene test: This test was performed in this study to test for homogeneity of variance of two sets of data to determine if a paired t-test was viable.

Paired t-test: see Section 2.2.5.6

Analysis of Variance (ANOVA): see Section 2.2.5.6

Wilcoxon Signed ranks test: see Section 3.2.3.1

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[1] values are in the appendices of Putman *et al* (1981), this citation refers to these appendices throughout

4.3 RESULTS

4.3.1 Condition of samples

All of the samples of vegetation were five years old when chemical analysis was carried out. Although they had been dried and stored correctly in sealed glass vials, it was felt that their nutrient content may have altered. A comparison between 'old' and 'new' vegetation was necessary. Five species of fresh plant material ('new') were selected (Ivy, Holly, Bramble, *Calluna vulgaris* and *Polytrichum* spp.) and collected from Brinken, they were milled, dried and stored (for one month) in the same way as the original samples. Their nutrient content was then measured and compared to the nutrient content of the same species collected at the same place exactly five years prior ('old'). Both 'old' and 'new' samples were analysed on the same day to avoid any errors associated with the equipment.

The two sets of data were tested for normality using Levene's test for equality of variances. Each test for each nutrient produced a non significant result suggesting that there was no evidence that the variances were unequal. Homogeneous variances are one of the data requirements for using paired t-tests and as these data fulfil those requirements this was performed to test the following null hypothesis:-

H_0 = there was no significant difference in the nutrient content of five year old and fresh vegetation.

The paired t-test showed that there were no significant differences between the 'old' and 'new' samples (nitrogen: $t_4 = 1.419$, NS; calcium: $t_4 = 1.918$, NS; magnesium: $t_4 = 1.665$, NS; potassium: $t_4 = 2.255$, NS; phosphorus: $t_4 = -2.047$, NS). The null hypothesis was accepted in each case. Therefore the age of the vegetation that was used in subsequent chemical analysis and to which the faecal data would be related (Chapter 5), would not compromise the nutrient profiles of each species of vegetation analysed.

4.3.2 Reduction of samples

There were logistical, financial and time constraints on the number of samples that could be analysed. It was imperative to determine if the sample number could be reduced.

Where possible plant samples had been collected every month for a consecutive 12 month period at each site. As the faecal data had been analysed using information for bimonth rather than separate months, it was appropriate to use bimonth data for the plant samples. Whereas the bimonth data for the faecal analysis comprised of data from each respective month, in order to reduce the number of plant samples examined, statistical analysis was performed to determine if just the first month of each bimonth could be used to represent the bimonth.

Eight samples of vegetation were chosen, two from each site and chemical analyses were carried out for each month creating a complete year's nutrient profile for the selected plants. The plants were chosen on the basis that most major groups of vegetation were represented (*e.g.* Conifer, Herb, Monocotyledon) eaten by both fallow and roe deer (Chapter 2) and were those that had been collected for every month (Table 4.1). The nutrient data were divided into the two following sets of months:

A	Jan	Mar	May	Jul	Sep	Nov
B	Feb	Apr	Jun	Aug	Oct	Dec

Table 4.1 Plant species selected (and the group they represented) to determine if a reduction in the number of samples for chemical analysis was possible.

Vegetation Sample	Group represented	Site
Bramble		Brinken
Ivy		Brinken
Douglas fir	Conifer	Denny Lodge
Juncus spp.	Monocotyledon	Denny Lodge
Calluna vulgaris	Dwarf shrub	Great Covert
Wood sage	Herb	Great Covert
Holcus lanatus	Grass/Monocotyledon	Squabb
Western hemlock	Conifer	Squabb

The data for each corresponding month for a bimonth from A and B (*e.g.* Jan and Feb) were tested for normality using Levene's test for equality of variance for each element in turn. Potassium produced significant results for March-April ($F_{14} = 6.008$, $P<0.05$) and May-June ($F_{14} = 10.422$, $P<0.01$), calcium for September-October ($F_{14} = 10.34$, $P<0.01$) and magnesium also for September-October ($F_{14} = 4.943$, $P<0.05$). Therefore as not all combinations showed homogeneous variances a Wilcoxon signed ranks test was performed which is less powerful than a paired t-test, but makes fewer assumptions about the shape of the data (Dytham, 1999). The following null hypothesis was tested:-

H_0 = there was no significant difference in the nutrient content of each sample taken from the first and second month of a bimonth.

For each nutrient and each bimonth combination there was no significant difference between the nutrient content for each month of the pair. This suggests that taking one month to represent the nutrient content of the bimonth was acceptable.

The months underlined in the following list of bimonths were those analysed.

JanFeb MarApr MayJun JulAug SepOct NovDec

Where a sample of vegetation had not been collected in the required month but had been in its partner month this was analysed in its place (54 of the 501 samples).

4.3.3 Composite groups

The faecal analysis presented several groups of vegetation where individual plant species could not be recognised so a higher classification was used. The groups were Herbs, Grasses (Monocotyledons) and Deciduous trees (Appendix 2). In order to contrast the quantities in which these groups were eaten by the fallow and roe deer with the quality, *i.e.* the nutrient content, (Chapter 5), it was necessary to produce mean bimonth values for each group.

4.3.3.1 Herbs

Ideally, to obtain a mean nutrient content for the group Herb would have been to chemically analyse several different Herbs over the six bimonths for each site, however this was not possible due to the financial and time constraints of the chemical analysis. From the faecal analysis the group Herb was likely to have consisted of several different plants, none of which were identified to species level. To obtain a mean bimonthly nutrient content for this group, six Herbs were analysed and considered as replicates. Each Herb was represented at two of the four sites with each site providing three different species of plant. In some bimonths, notably during winter, many of the Herbs were found in very small quantities, in such cases the material was analysed for nitrogen alone as this was the nutrient believed to be of most importance in forage selection by deer (Parfitt, in Putman, 1986a). Otherwise each species was analysed for all the nutrient components. The data were transformed using arcsine square root, acceptable for data measuring proportions (Dytham, 1999), and then examined using ANOVA. In this analysis the interaction term site*bimonth was of main interest. The null hypothesis was:

H_0 = there was no significant difference between the sites over the six bimonths

If this hypothesis was accepted then a mean for each bimonth could be calculated as their nutrient content did not differ significantly across the sites.

From Table 4.2 it is evident for nitrogen, magnesium, potassium and phosphorus that a mean value for Herb can be taken for each bimonth and that these can represent all four sites. However, for calcium the null hypothesis could not be accepted therefore means cannot be relied upon for this nutrient.

Table 4.2 ANOVA results (F values and probability levels) of the effects of the interaction term bimonth*site for the different nutrient components of the group Herb (NS= not significant).

Nutrient	**Bimonth*Site**
Nitrogen	$F_{15,36} = 0.437$, NS
Calcium	$F_{15,17} = 2.444$, $P<0.05$
Magnesium	$F_{15,17} = 1.016$, NS
Potassium	$F_{15,17} = 0.62$, NS
Phosphorus	$F_{15,17} = 0.27$, NS

4.3.3.2 Grasses

At each site most species of grass were individually identified in the diet (where this was not possible they were labelled as the group Grass), as a result they were individually chemically analysed. Therefore for each site a mean bimonth value for each nutrient was taken from all the individual grasses analysed. This was also done for the subgroups of Coarse and Sweet Grasses (Appendix 2).

4.3.3.3 Deciduous tree

Mean values for the nutrient content of the Deciduous tree group were calculated in the same way as Grasses. Each site was considered separately and means for each bimonth were taken from the nutrient content of all deciduous trees (Appendix 2).

4.3.4 Trends in the quality of vegetation at each site

No replication was possible at each site for the chemical analysis of each plant sample. This gave little scope for statistical analysis and most of the data analysis is therefore descriptive. The pattern of the content of each nutrient over the year was however investigated using one-way ANOVA. As this is a parametric test the data were transformed using the arcsine square root transformation. This test used all of the nutrient data from individual plant species prior to forming composite groups. Collection of samples had not been consistent over the year for many reasons, *e.g.* seasonality of some plants, therefore sample sizes for each bimonth were different.

The intention was to investigate general seasonal patterns of each nutrient, this could be done using all the data across all sites, however one-way ANOVA showed there were significant differences between the sites for calcium ($F_{3,378} = 8.729$, $P<0.001$), potassium ($F_{3,378} = 16.42$, $P<0.001$) and phosphorus ($F_{3,378} = 3.062$, $P<0.05$). Multiple range tests indicated that the fallow sites (Brinken and Denny Lodge) were significantly different to the roe sites (Great Covert and Squabb). Moreover, the two fallow sites were significantly different from each other for calcium and potassium. In view of this, the seasonal variation at each site was compared to the published data. This in turn allowed the reliability of the analytical techniques to be determined.

4.3.4.1 Fallow deer sites

Brinken

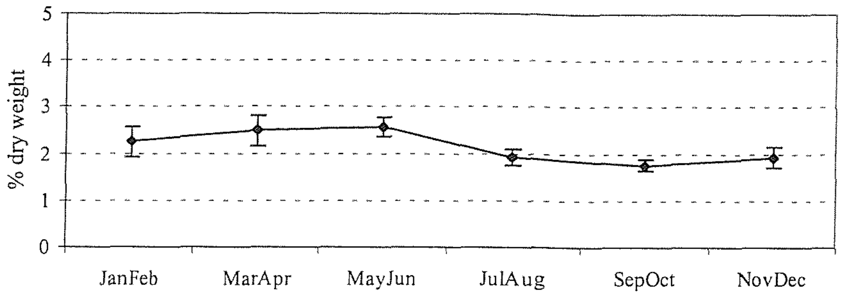
There was a significant effect of bimonth (at P<0.1 level) on the **nitrogen** content of the vegetation ($F_{5,80}$ = 1.965, P<0.1). A post hoc multiple range test suggested that this effect was largely due to the difference in nitrogen content between the bimonths SepOct and MayJun (Figure 4.1a), vegetation from the former having the lowest nitrogen content (1.76% dry weight) and the latter having the highest (2.57%). Figure 4.1b shows the mean **calcium** content for each bimonth over the year. There was very little variation and the ANOVA showed no significant effect of bimonth ($F_{5,86}$ = 0.587, NS). Similarly there was no significant effect of bimonth on **magnesium** content of the plant species eaten ($F_{5,86}$ = 1.742, NS). Although not significant the overall means showed a rise in MarApr, which was maintained into MayJun and then declined (Figure 4.1c). The **potassium** content of the vegetation was also not significantly affected by bimonth ($F_{5,86}$ = 0.645, NS). Figure 4.1d shows the mean potassium content gently rose in the spring and decreased in late summer. There was a significant effect of bimonth on the **phosphorus** content of the plant species eaten ($F_{5,87}$ = 2.911, P<0.05; Figure 4.1e). A post hoc multiple range test contrasted MayJun with JulAug, the former having a mean phosphorus content of 0.14% dry weight and the latter having the lowest, 0.07% dry weight. This significant decline occurred in a relatively short space of time.

Denny Lodge

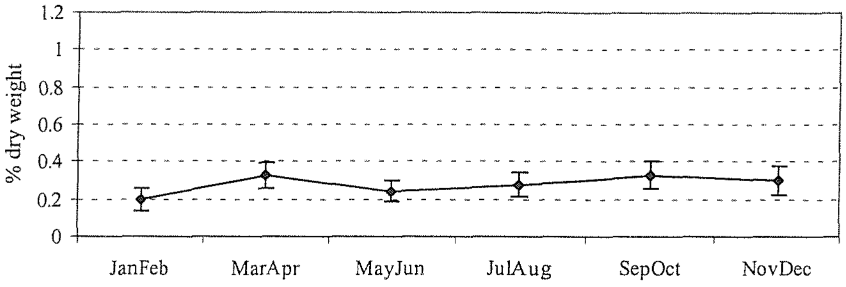
At this site there was a much stronger effect of bimonth ($F_{5,108}$ = 4.386, P=0.001) on the **nitrogen** content compared that at Brinken. Here the multiple range test showed that there was a clear division of the year into two halves. At Denny Lodge JanFeb, NovDec and SepOct (lowest nitrogen content first) were contrasted against MayJun and MarApr (highest nitrogen content last), with JulAug not differing from either group (Figure 4.2a). There was no significant effect of bimonth for the **calcium** content of vegetation ($F_{5,114}$ = 0.427, NS). The means varied very little over the year (Figure 4.2b). There was similarly no significant effect of bimonth on the **magnesium** content ($F_{5,114}$ = 0.279, NS). The means show that the winter was the period of lowest magnesium (Figure 4.2c) despite not being significantly different to the rest of the year. At the P<0.1 level there was a significant effect of bimonth on the content of **potassium** in the vegetation eaten ($F_{5,114}$ = 1.938, P<0.1). A multiple range test indicated that the main difference was between the summer months (MayJun, JulAug) and JanFeb. The summer months contained a mean of 2.22% and 2.13% dry weight respectively and

Figure 4.1 Mean bimonth nutrient content (± standard error) of all plant species analysed from Brinken.

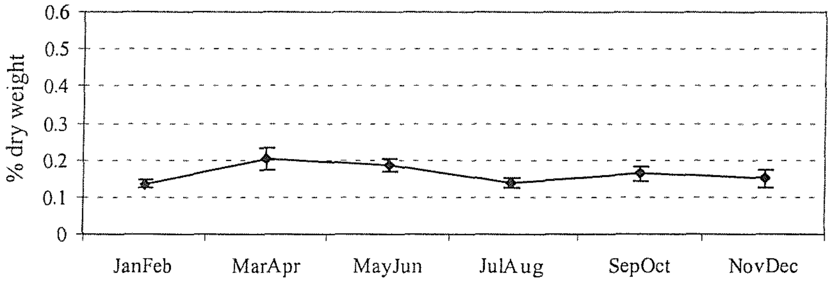
a) Nitrogen



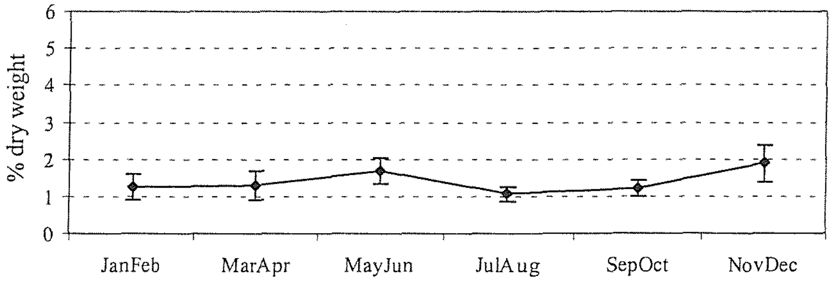
b) Calcium



c) Magnesium



d) Potassium



e) Phosphorus

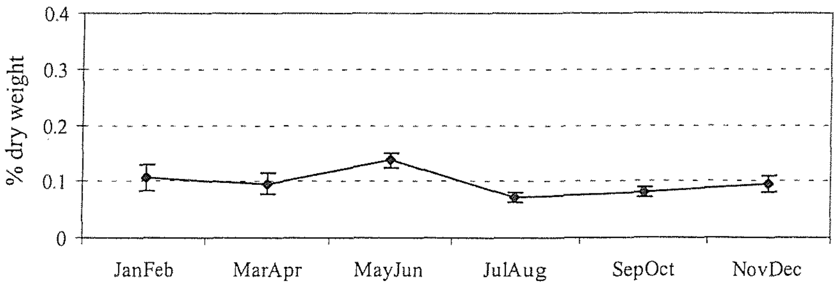
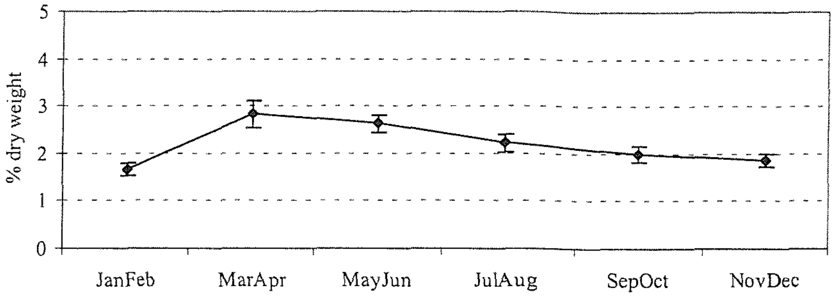
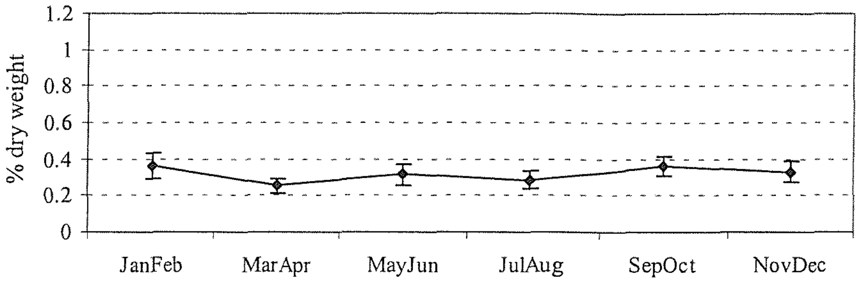


Figure 4.2 Mean bimonth nutrient content (± standard error) of all plant species analysed from Denny Lodge.

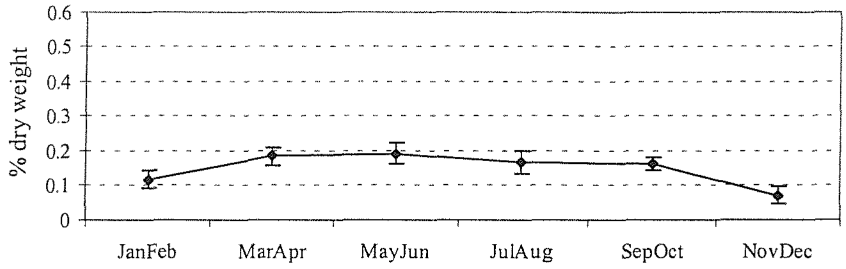
a) Nitrogen



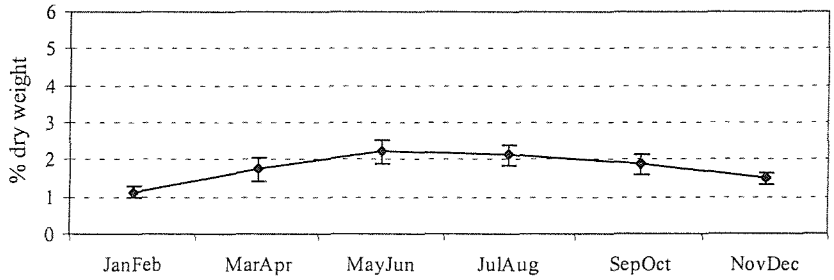
b) Calcium



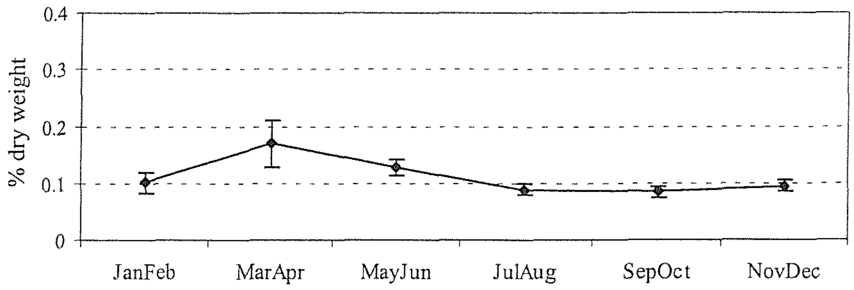
c) Magnesium



d) Potassium



e) Phosphorus



JanFeb contained only 1.13% dry weight (Figure 4.2d). A significant effect of bimonth was also found for **phosphorus** content ($F_{5,114} = 2.537$, $P<0.05$), the multiple range test contrasted MarApr (0.17% dry weight) with the remaining bimonths. Figure 4.2e shows the large error bars for MarApr suggesting larger variation in phosphorus content in this bimonth than any other, therefore this significant result could be an anomaly.

4.3.4.2 Roe deer sites

Great Covert

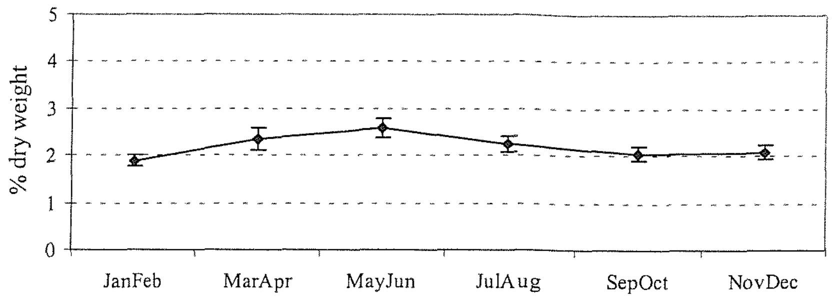
For **nitrogen** content at Great Covert there was no significant effect of bimonth when all the vegetation analysed were considered ($F_{5,116} = 1.644$, NS). This is clearly reflected in the means shown in Figure 4.3a. Also, no significant effect of bimonth was found for the **calcium** ($F_{5,119} = 0.192$, NS), **magnesium** ($F_{5,119} = 1.599$, NS), **potassium** ($F_{5,119} = 1.412$, NS) or **phosphorus** ($F_{5,119} = 1.206$, NS) content (Figures 4.3b - e). Figure 4.3c shows the mean magnesium content over the year varied very little and this explains the lack of a significant effect for bimonth. There was also very little variation in the phosphorus content over the year (Figure 4.3e) apart from a decline in JanFeb, but this was insufficient to produce a significant result even at the $P<0.1$ level.

Squabb

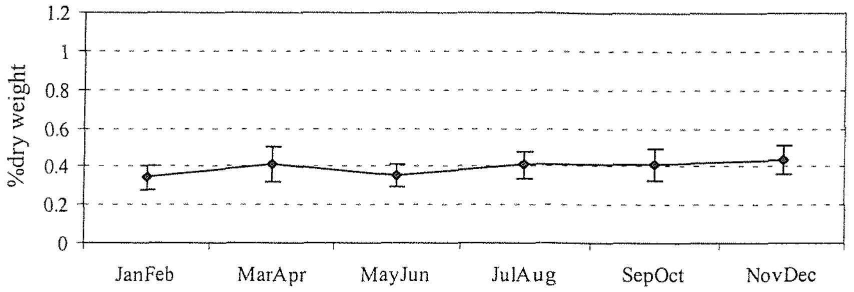
Squabb, as for the other roe site, did not show any significant effect of bimonth for **nitrogen** content ($F_{5,106} = 0.679$, NS) of all the vegetation analysed. This is shown by the overall bimonth means in Figure 4.4a. The levels here were even more consistent than at Great Covert. Over the year there was little variation in the mean content of **calcium** (Figure 4.4b) hence there was no significant effect of bimonth for this nutrient ($F_{5,117} = 0.802$, NS). The means however suggested a decline in the summer months. There was also no significant effect of bimonth on the **magnesium** content of the vegetation eaten ($F_{5,117} = 0.806$, NS; Figure 4.4c). Although the means showed a gentle increase in **potassium** content into the summer and decrease into the winter (Figure 4.4d) there was no significant effect of bimonth ($F_{5,117} = 1.298$, NS). Figure 4.4e shows hardly any variation in the **phosphorus** content means across the year and this is supported by no significant effect of bimonth ($F_{5,117} = 0.19$, NS).

Figure 4.3 Mean bimonth nutrient content (± standard error) of all plant species analysed from Great Covert.

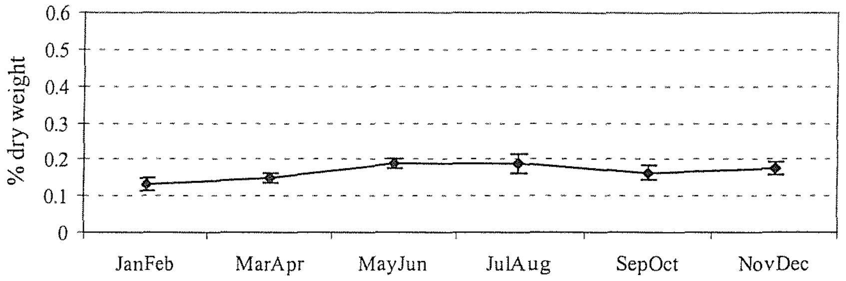
a) Nitrogen



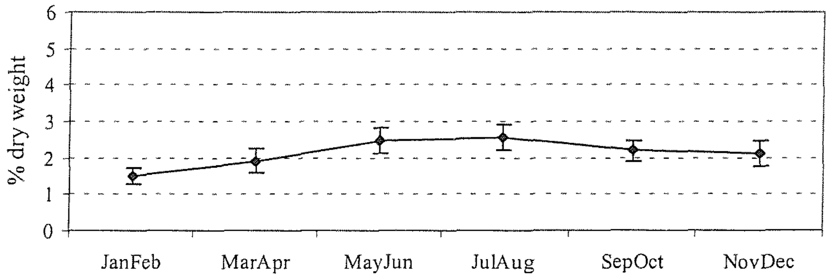
b) Calcium



c) Magnesium



d) Potassium



e) Phosphorus

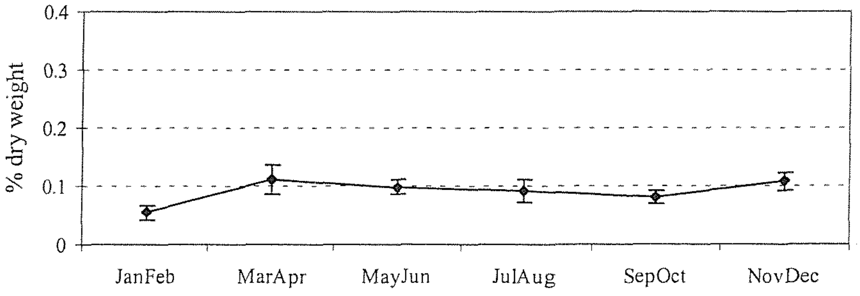
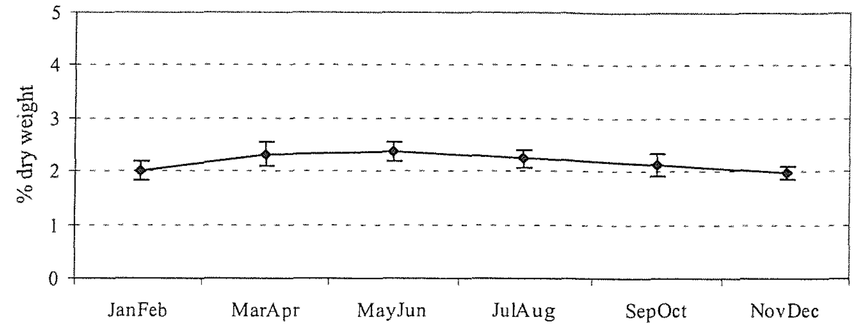
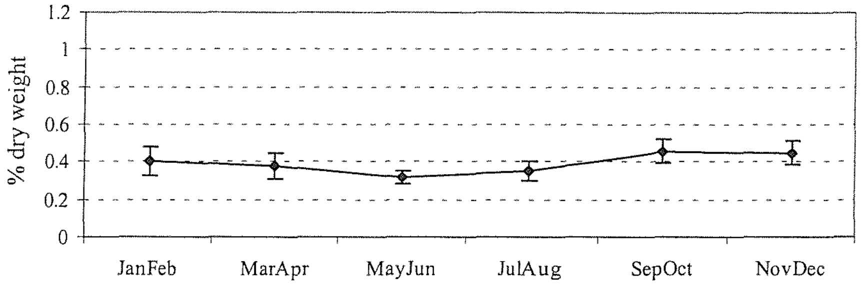


Figure 4.4 Mean bimonth nutrient content (± standard error) of all plant species analysed from Squabb.

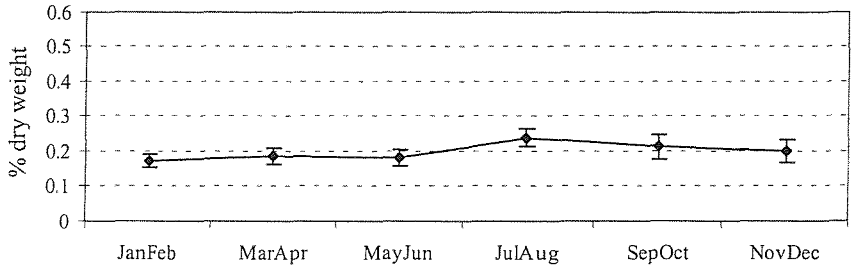
a) Nitrogen



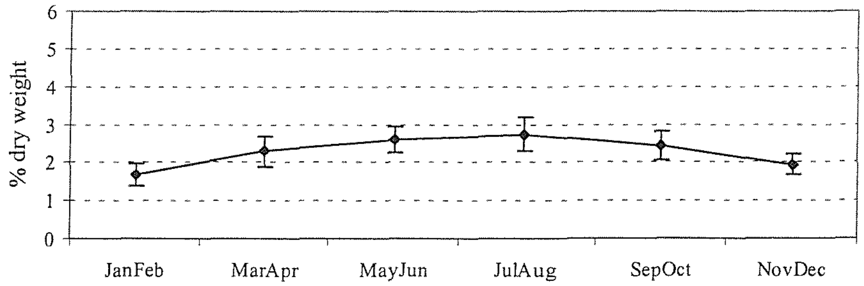
b) Calcium



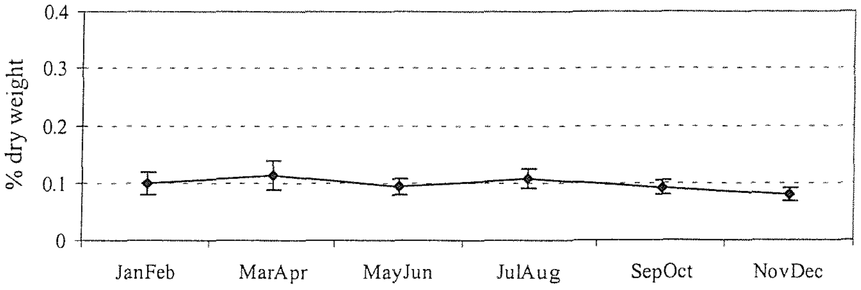
c) Magnesium



d) Potassium



e) Phosphorus



4.3.5 Trends in the quality of the important forage species.

This analysis, although similar to the previous section, was designed to look specifically at the important plant species in the diet. It provided more detailed analysis and information on individual species across the four sites. Moreover, this analysis may help to explain diet selection.

The sample size was reduced to the twelve plant species considered to be important across the sites as most occurred as ≥5% of the diet (Table 4.3). It should however be noted that they differed in their importance at each site and not all were found at Brinken, Table 4.3 shows the plant species analysed.

Table 4.3 Plant samples chosen for chemical analysis and their proportion in the diet.

	Brinken	**Denny Lodge**	**Great Covert**	**Squabb**
Holly	***	***	□	□
Bramble	***	□	***	***
Ivy	***	**	***	***
Polytrichum spp.	***	***	**	***
Oak	***	***	***	***
Birch	**	*	*	***
Western hemlock		*	***	**
Douglas fir		□	***	□
Pine	(***)	***	**	***
Carex spp.	**	***	**	**
Juncus spp.	(**)	***	□	***
Bracken	**	*	**	***

Legend:	
***	≥5% of the diet and collected for chemical analysis (brackets denote **not** found)
**	≥2% of the diet and collected for chemical analysis (brackets denote **not** found)
*	≥1% but <2% of the diet and collected for chemical analysis
□	not present in diet but collected for chemical analysis
blank cells	vegetation not eaten and not collected for chemical analysis

The data were transformed using the arcsine square root transformation and a two-way ANOVA was carried out. Bimonth (seasonal variation), site (inter-site) and plant species (interspecific) were the main effects with plant species*bimonth and plant species*site as the interactions terms. Post hoc multiple range (Duncan) tests were performed when the main effects were significant. As this was the case, F values and probability levels for significant main effects are reported in addition to the significant interaction terms. Each nutrient will be considered separately. Most of the forage species (Holly, Bramble, Oak, *Juncus* spp., *Polytrichum* spp., Bracken and Pine) important in this study have been chemically analysed by Ekins (1989) who also worked in the New Forest. Appendix 8 compares Ekins' (1989) findings with the current study.

4.3.5.1 Nitrogen

Interspecific comparison

Nitrogen content was significantly different between plant species ($F_{11,130} = 20.777$, $P<0.001$). Figure 4.5 shows the plant species in order of highest to lowest mean nitrogen content. A post hoc multiple range test created several significantly different groups within these twelve species of vegetation. The most notable contrasts were deciduous trees (Birch and Oak) and evergreens (including Western hemlock, Douglas fir, Holly, Ivy and *Polytrichum* spp.; Figure 4.5).

Seasonal variation

Nitrogen content was significantly affected by bimonth ($F_{5,130} = 9.243$, $P<0.001$; Figure 4.6), the multiple range test showed that the year was split into autumn/winter and spring/summer but it largely contrasted JanFeb (1.64% dry weight) with MayJun (2.31%). The interaction term plant species*bimonth was significant ($F_{52,130} = 3.239$, $P<0.001$), indicating that the twelve plant species differed in their nitrogen content over the year.

Inter-site comparison

The main effect of site was significant ($F_{3,130} = 7.345$, $P<0.001$) and the multiple range test contrasted Great Covert with the highest mean nitrogen content (2.06%) against the two fallow sites with Squabb falling in between and not differing significantly from either. Figure 4.7 shows the differences between the sites were in fact very small. Plant species*site was also significant ($F_{29,130} = 2.709$, $P<0.001$) indicating that the different plant species have different amounts of nitrogen at the four different sites.

Figure 4.5 Mean nitrogen content (± standard error) for each plant species (ANOVA: $F_{11,130}$ = 20.777, P<0.001). Multiple range test (Duncan) clusters shown by solid lines. (Birch N=19; Oak N=14; Bramble N=24; *Carex* spp. N=24; Bracken N=24; *Juncus* spp. N=18; Pine N=16; *Polytrichum* spp. N=24; Ivy N=23; Holly N=24; Douglas fir N=18; Western hemlock N=18).

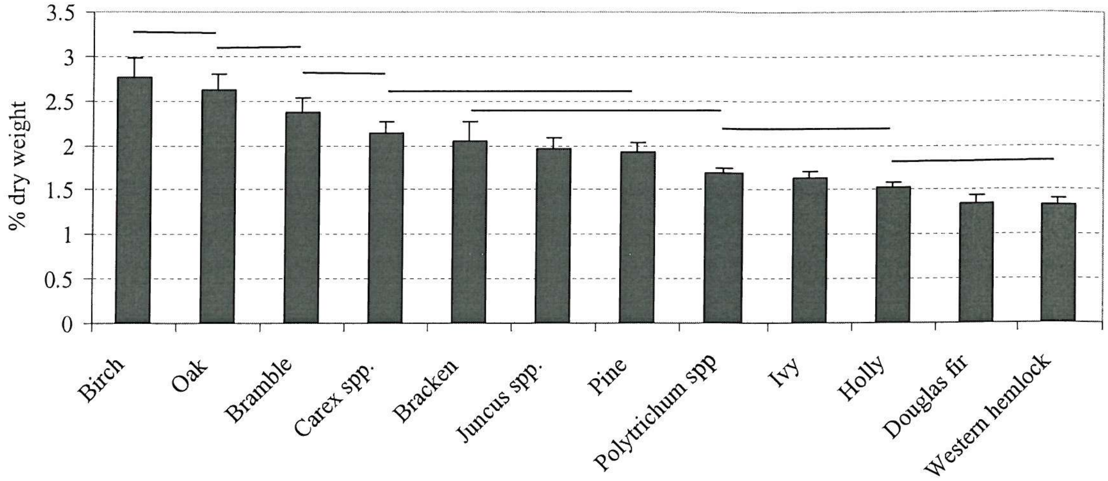
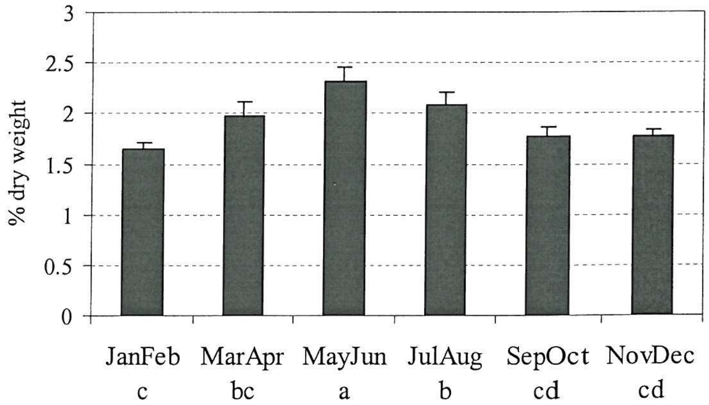
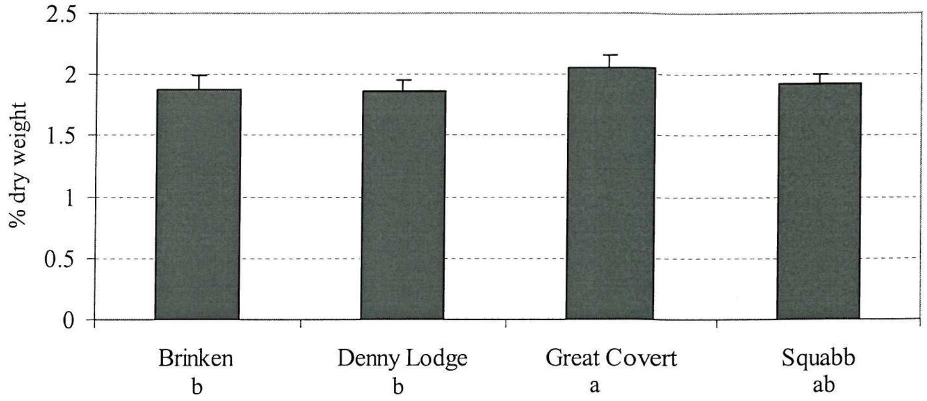


Figure 4.6 Mean nitrogen content (± standard error) for each bimonth using data from twelve plant species common to all four sites (ANOVA: $F_{5,130}$ = 9.243, P<0.001; JanFeb N=36; MarApr N=39; MayJun N=44; JulAug N=43; SepOct N=43 NovDec N=41).



[**N.B.** The bimonths were kept in time order, therefore the results from the multiple range test could not indicated as in Figure 4.5. In this case the same letter after the bimonth on the x axis denotes no significant difference. This method will be used for Figures 4.6, 7, 9, 10, 13, 15, 16, 19]

Figure 4.7 Mean nitrogen content (± standard error) for each site using data from twelve plant species common to all four sites (ANOVA: $F_{3,130} = 7.345$, $P<0.001$; Brinken N=43; Denny Lodge N=67; Great Covert N=67; Squabb N=69)



4.3.5.2 Calcium

Interspecific comparison

Plant species varied significantly in their calcium content ($F_{11,129} = 62.109$, $P<0.001$; Figure 4.8). A multiple range test produced different clusters of the vegetation and these are shown in Figure 4.8. The most significantly different species from each other were Ivy, Holly and *Polytrichum* spp. The remaining species cross over into several different clusters. Apart from *Juncus* spp. and *Carex* spp. forming one group as Monocotyledons, there does not appear to be a pattern with the other clusters. The conifers (Pine, Western hemlock and Douglas fir) were clustered together but with Birch.

Seasonal variation

Calcium content was significantly affected by bimonth ($F_{5,129} = 5.749$, $P<0.001$; Figure 4.9). A multiple range test highlighted that in particular, NovDec, JanFeb and SepOct were significantly different to JulAug and MayJun. This suggests a marked division between autumn/winter and summer as found for nitrogen. However, in the case of calcium the highest content was in autumn/winter (0.39-0.42% dry weight) and the lowest was in summer (0.3-0.32%), an inversed pattern to that seen in nitrogen. The interaction term, plant species*bimonth was significant for calcium ($F_{52,129} = 2.984$, $P<0.001$) indicating that although the general trend was a decrease in the summer bimonths, different species of vegetation show different patterns of calcium content over the year.

Figure 4.8 Mean calcium content (± standard error) for each plant species (ANOVA: $F_{11,129}$ = 62.109, P<0.001). Multiple range test (Duncan) clusters shown by solid lines. (Birch N=19; Oak N=14; Bramble N=24; *Carex* spp. N=23; Bracken N=24; *Juncus* spp. N=18; Pine N=16; *Polytrichum* spp. N=24; Ivy N=23; Holly N=24; Douglas fir N=18; Western hemlock N=18).

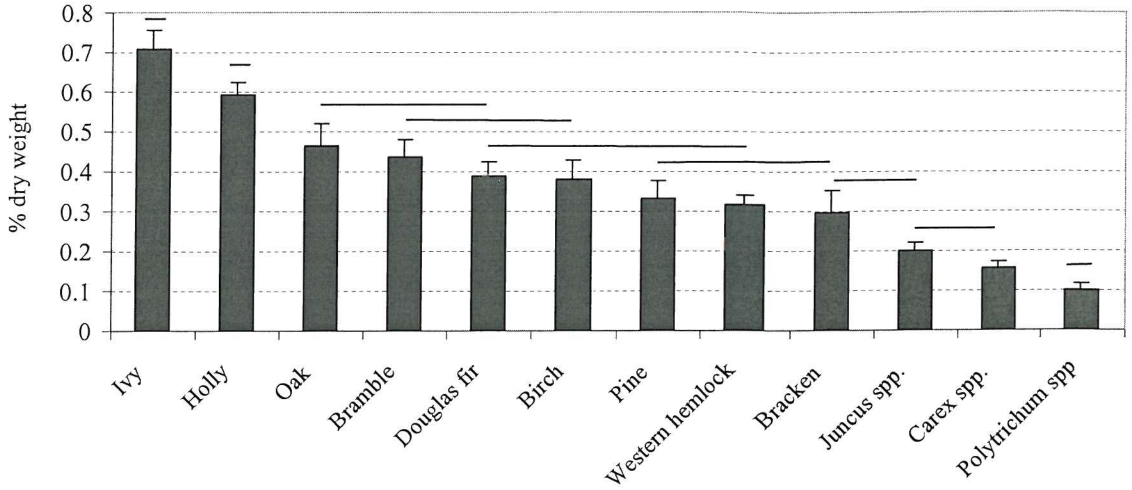
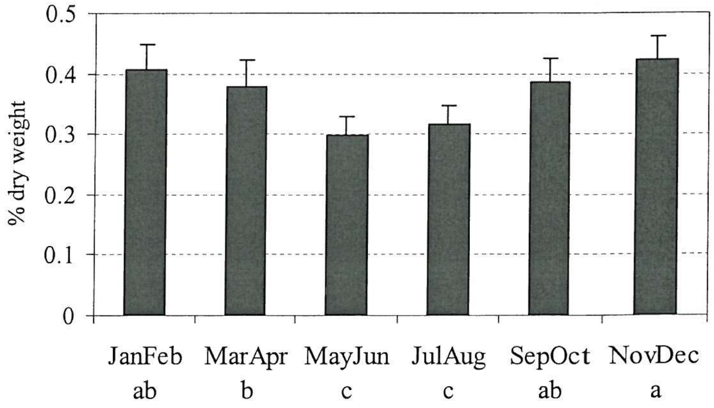


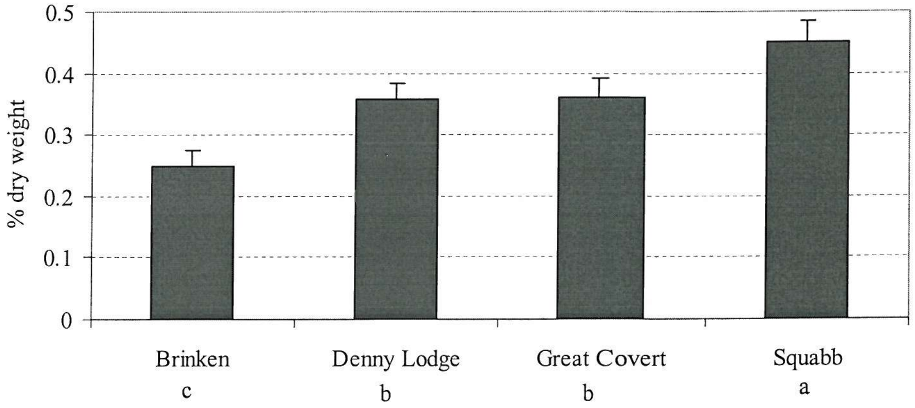
Figure 4.9 Mean calcium content (± standard error) for each bimonth using data from twelve plant species common to all four sites (ANOVA: $F_{5,129}$ = 5.749, P<0.001; JanFeb N=36; MarApr N=39; MayJun N=44; JulAug N=43; SepOct N=43 NovDec N=40).



Inter-site comparison

The calcium content was significantly affected by site ($F_{3,129} = 36.756$, $P<0.001$; Figure 4.10), the main contrast was between Squabb (0.45% dry weight) and Brinken (0.248% dry weight) as shown by the multiple range test. Denny Lodge and Great Covert were clustered in between the two extremes. A significant interaction between plant species and site was also found ($F_{29,129} = 2.952$, $P<0.001$): the plant species at the different sites contain different amounts of calcium.

Figure 4.10 Mean calcium content (± standard error) for each site using data from twelve plant species common to all four sites (ANOVA: $F_{3,129} = 36.756$, $P<0.001$; Brinken N=42; Denny Lodge N=67; Great Covert N=67; Squabb N=69)



4.3.5.3 Magnesium

Interspecific comparison

Magnesium content differed significantly between plant species ($F_{11,129} = 28.817$, $P<0.001$). Figure 4.11 shows that this was likely to be driven by the high mean magnesium content of Bramble which was significantly different from all the other species in the multiple range test. *Polytrichum* spp. was also significantly different from the remaining species which formed three clusters. As for calcium, the conifers were coupled with Birch.

Seasonal variation

There was no significant effect of bimonth on the magnesium content ($F_{5,129} = 1.148$, NS). Figure 4.12 shows that apart from a slight rise in JulAug, the level was very similar throughout the year.

Figure 4.11 Mean magnesium content (± standard error) for each plant species (ANOVA: $F_{11,129} = 28.817$, $P<0.001$). Multiple range test (Duncan) clusters shown by solid lines. (Birch N=19; Oak N=14; Bramble N=24; *Carex* spp. N=23; Bracken N=24; *Juncus* spp. N=18; Pine N=16; *Polytrichum* spp. N=24; Ivy N=23; Holly N=24; Douglas fir N=18; Western hemlock N=18).

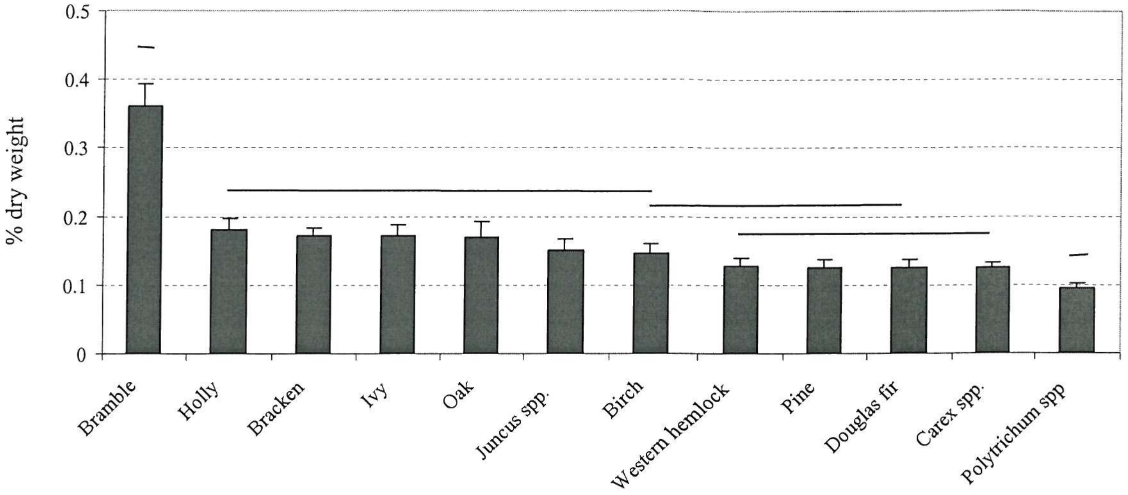
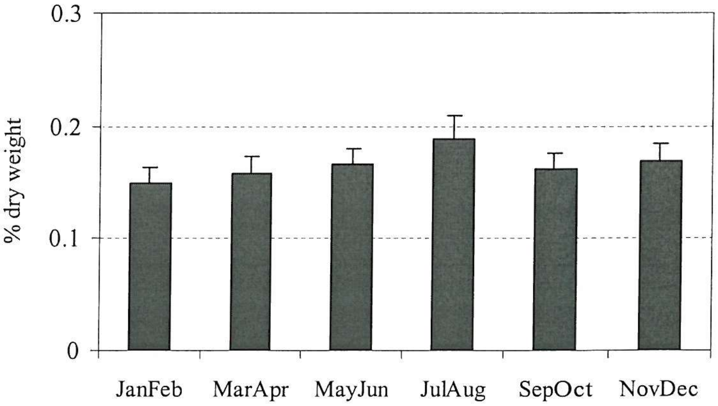


Figure 4.12 Mean magnesium content (± standard error) for each bimonth using data from twelve plant species common to all four sites (ANOVA: $F_{5,129} = 1.148$, NS; JanFeb N=36; MarApr N=39; MayJun N=44; JulAug N=43; SepOct N=43 NovDec N=40).

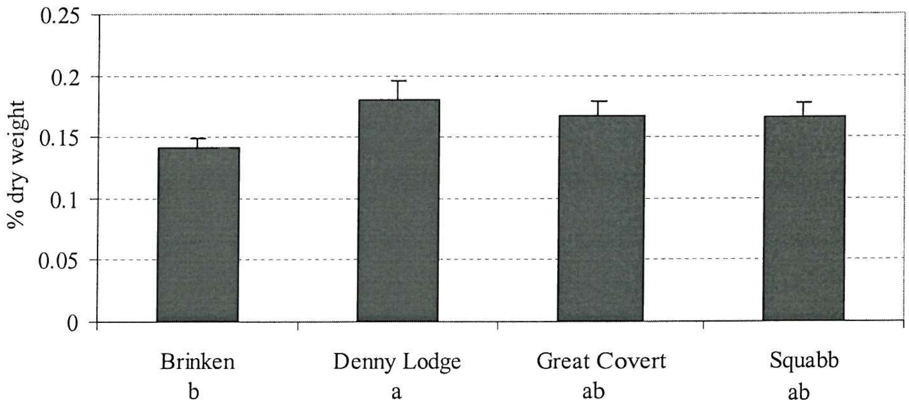


Although there was no significant effect of bimonth, the interaction between bimonth and plant species was significant for this nutrient ($F_{52,129} = 1.96$, P=0.001).

Inter-site comparison

Magnesium content was significantly affected by site ($F_{3,129} = 5.393$, P<0.005; Figure 4.13). The multiple range test showed that the main differences were between the two fallow sites, Denny Lodge (0.18% dry weight) and Brinken (0.14%). The two roe sites fell in between the fallow sites with respect to their magnesium content. The plant species*site interaction was significant for magnesium ($F_{29,129} = 3.7$, P<0.001). Thus as for nitrogen and calcium, the plants contained different levels of magnesium at each site.

Figure 4.13 Mean magnesium content (± standard error) for each site using data from twelve plant species common to all four sites (ANOVA: $F_{3,129} = 5.393$, P<0.005; Brinken N=42; Denny Lodge N=67; Great Covert N=67; Squabb N=69)



4.3.5.4 Potassium

Interspecific comparison

Potassium content was significantly different between the plant species ($F_{11,129} = 444.144$, P<0.001). The multiple range test produced several significantly different clusters (Figure 4.14), the biggest contrast was between the monocotyledon (*Carex* spp. and *Juncus* spp.) and conifer clusters (Pine, Western hemlock and Douglas fir) with *Polytrichum* spp.

Figure 4.14 Mean potassium content (± standard error) for each plant species (ANOVA: $F_{11,129}$ = 44.144, P<0.001). Multiple range test (Duncan) clusters shown by solid lines. (Birch N=19; Oak N=14; Bramble N=24; *Carex* spp. N=23; Bracken N=24; *Juncus* spp. N=18; Pine N=16; *Polytrichum* spp. N=24; Ivy N=23; Holly N=24; Douglas fir N=18; Western hemlock N=18).

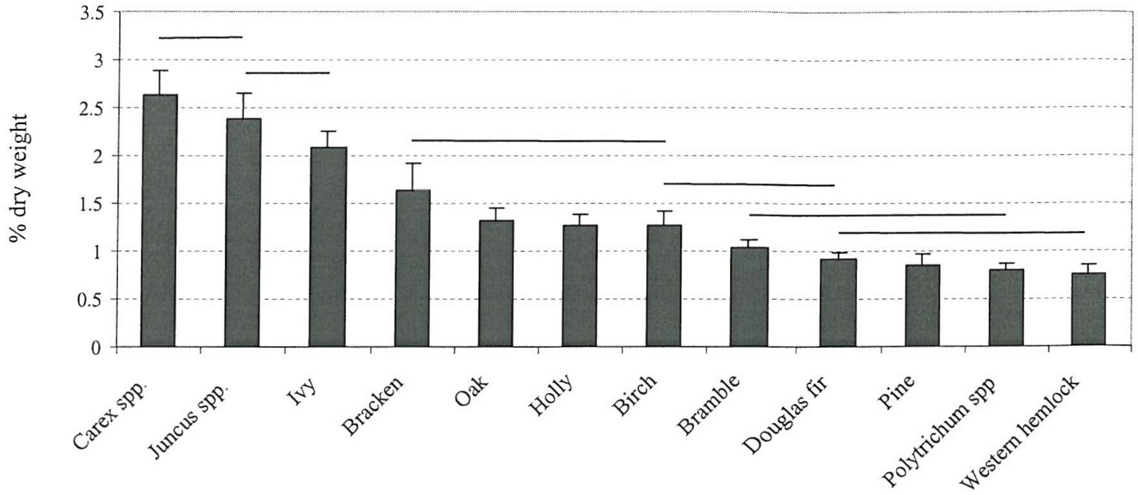
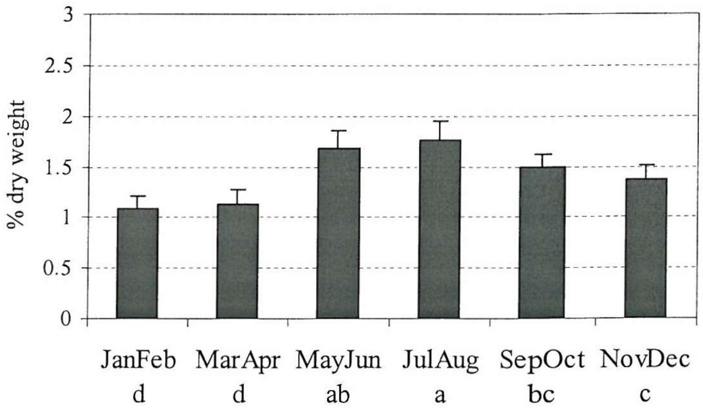


Figure 4.15 Mean potassium content (± standard error) for each bimonth using data from twelve plant species common to all four sites (ANOVA: $F_{5,129}$ = 15.481, P<0.001; JanFeb N=36; MarApr N=39; MayJun N=44; JulAug N=43; SepOct N=43 NovDec N=40).



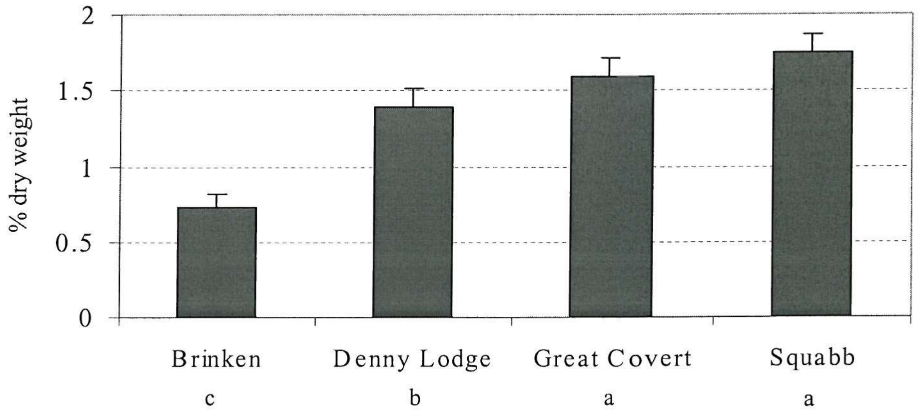
Seasonal variation

Potassium content was significantly affected by bimonth ($F_{5,129}$ = 15.481, P<0.001). The multiple range test particularly contrasts JulAug and MayJun with MarApr and JanFeb (Figure 4.15) with the latter pair demonstrating the lowest amounts of potassium (1.08-1.13% dry weight). This comparison suggests that the potassium content of plants was at its most different between late winter/spring and the summer. This pattern was similar to nitrogen and is opposite to calcium. For potassium the interaction between plant species and bimonth was significant ($F_{52,129}$ = 4.07, P<0.001). Therefore as with nitrogen, although there is a suggested trend of increased potassium content in the summer and decrease in the winter, many individual plant species show different patterns over the year .

Inter-site comparison

There was a significant effect of site on the potassium content of vegetation eaten ($F_{3,129}$ = 68.307, P<0.001). Figure 4.16 shows very clearly why this was the case, vegetation at Brinken had a very low overall potassium content (0.73% dry weight) and the multiple range test contrasted this site against the others, however the roe sites were also pulled out as one group as they had the highest potassium content. The interaction between plant species and site was also significant for potassium ($F_{29,129}$ = 3.388, P<0.001), as plants at each site contained different amounts of potassium

Figure 4.16 Mean potassium content (± standard error) for each site using data from twelve plant species common to all four sites (ANOVA: $F_{3,129}$ = 68.307, P<0.001; Brinken N=42; Denny Lodge N=67; Great Covert N=67; Squabb N=69)



4.3.5.5 Phosphorus

Interspecific comparison

The phosphorus content of the plants species differed significantly ($F_{11,129} = 9.359$, $P<0.001$; Figure 4.17). The multiple range test divided the species into several different clusters without any obvious reasons based on plant type.

Seasonal variation

There was no significant effect of bimonth on the phosphorus content ($F_{5,129} = 1.064$, NS; Figure 4.18), although there was more variation over the year than for magnesium. The interaction term plant species*bimonth was not significant ($F_{5,129} = 0.936$, NS). As no effect of bimonth was found either, it suggested that for phosphorus not only was there very little variation over the year, but many plant species did not vary from each other.

Inter-site comparison

The phosphorus content was significantly affected by site, although to a lesser degree than the other nutrients ($F_{3,129} = 3.349$, $P<0.05$; Figure 4.19). The multiple range test, as for magnesium, contrasted the two fallow sites, Denny Lodge having the highest phosphorus content (0.1% dry weight) and Brinken the lowest (0.07%). The roe sites were similar to each other and fell in between the fallow sites. As for the previous nutrients the interaction between plant species and site was significant for phosphorus ($F_{29,129} = 3.668$, $P<0.001$), the plants contained different levels of phosphorus at the different sites.

Figure 4.17 Mean phosphorus content (± standard error) for each plant species (ANOVA: $F_{11,129} = 9.359$, $P<0.001$). Multiple range test (Duncan) clusters shown by solid lines. (Birch N=19; Oak N=14; Bramble N=24; *Carex* spp. N=23; Bracken N=24; *Juncus* spp. N=18; Pine N=16; *Polytrichum* spp. N=24; Ivy N=23; Holly N=24; Douglas fir N=18; Western hemlock N=18).

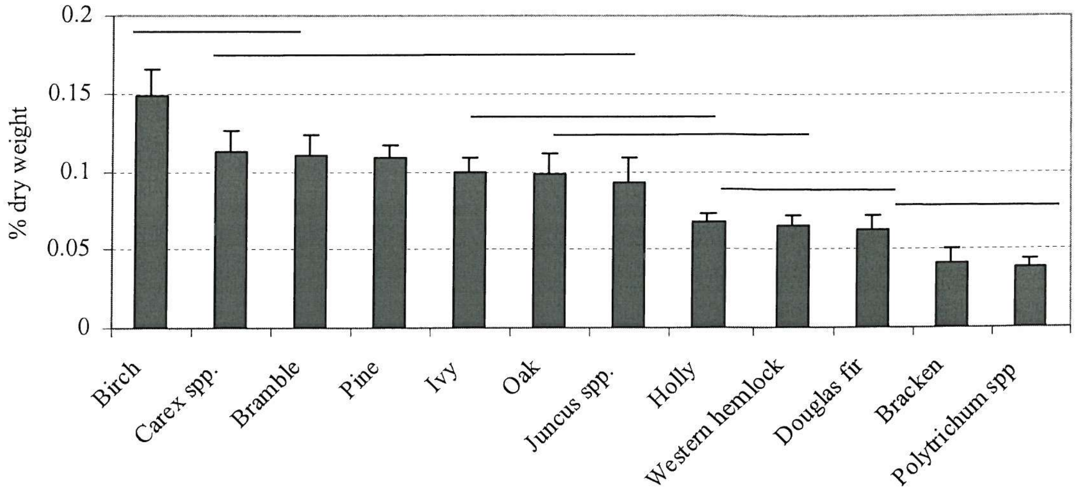


Figure 4.18 Mean phosphorus content (± standard error) for each bimonth using data from twelve plant species common to all four sites (ANOVA: $F_{5,129} = 1.064$, NS; JanFeb N=36; MarApr N=39; MayJun N=44; JulAug N=43; SepOct N=43 NovDec N=40).

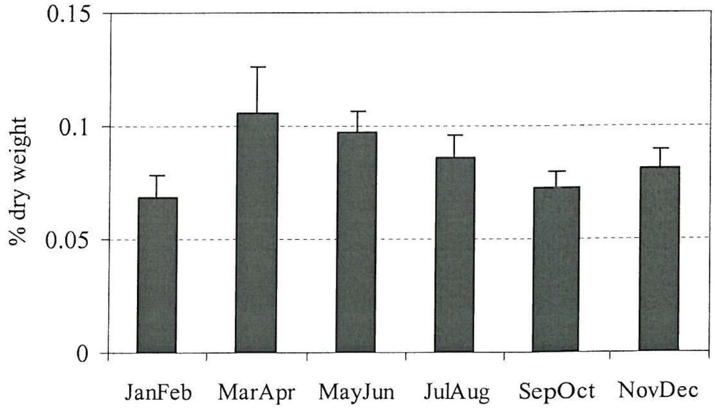
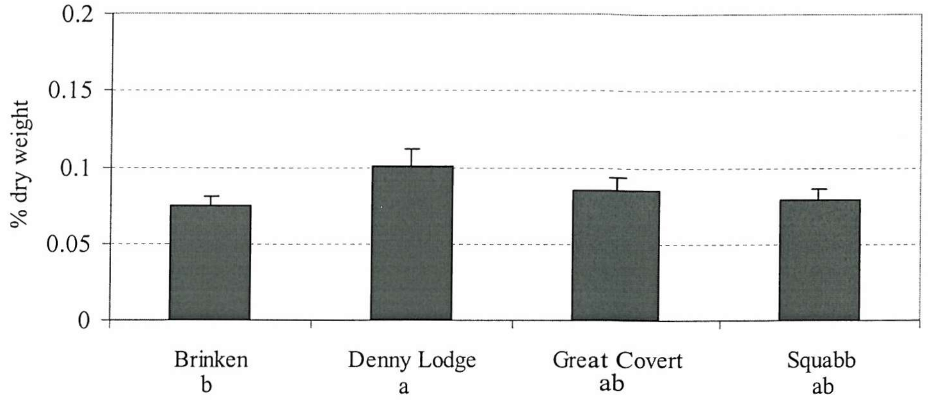


Figure 4.19 Mean phosphorus content (± standard error) for each site using data from twelve plant species common to all four sites (ANOVA: $F_{3,129} = 3.349$, $P<0.05$; Brinken N=42; Denny Lodge N=67; Great Covert N=67; Squabb N=69)



4.3.5.6 Digestibility

Dry matter digestibility values taken from Putman *et al* (1981) for the forage species important to the current study. N.B. *Carex* spp. were not included, Conifers were represented as a group and only one reading was taken to represent the whole year, this was also the case for Moss.

Table 4.4 Dry matter digestibility values reported by Putman *et al* (1981).

Plant species or group	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Birch	18.3	24.7	31.1	24.7	30.7	12
Oak	31.8	42.9	53.8	29	30.7	20.8
Holly	73.3	64.5	47.9	48.7	66.9	59
Conifers	20.5	20.5	20.5	20.5	20.5	20.5
Bramble	49.6	39.9	30.3	41	29.9	59.2
Ivy		69.6	62.3	73	69.4	84.1
Juncus spp.	22.5	21.5	31.5	21.6	22.1	24.1
Bracken	17.1	17.1	19.3	38.2	19.3	17.1
Moss	20.3	20.3	20.3	20.3	20.3	20.3

Summary

The twelve plant species showed significant differences from each other in terms of the nutrients they contained. There were similarities in the plant species that had the highest mean concentrations of nutrients and the lowest (Table 4.5).

Table 4.5 Plant species that contained significantly the most and least of each nutrient. Species in brackets were those in the top or bottom cluster produced by the multiple range test, but also cross into the next one (see Figures 4.5, 4.8, 4.11, 4.14, 4.17 for clusters).

	Nitrogen	Calcium	Magnesium	Potassium	Phosphorus
HIGH	Birch (Oak)	Ivy	Bramble	*Carex* spp. (*Juncus* spp.)	Birch (Bramble) (Pine)
LOW	Western hemlock (Douglas fir) (Holly)	*Polytrichum* spp	*Polytrichum* spp	Western hemlock (*Polytrichum* spp) Pine Douglas fir	*Polytrichum* spp Bracken (Douglas fir)

There was significant seasonal variation in the distribution of all the nutrients investigated. Most increased their concentration as the year went on, nitrogen and phosphorus in the spring, potassium in the summer and magnesium in late summer. Calcium showed an inverse distribution to the other nutrients, peaking in the winter months and decreasing through the spring and summer. There was also significant seasonal variation within the different plant species, these will be discussed in terms of Ekins' (1989) data (Section 4.4.2).

All the nutrients were significantly affected by site. In most cases, except nitrogen, Brinken contained the lowest amounts of nutrients from the twelve species analysed. For calcium and potassium this site was significantly different to the others. However, it was not significantly different to the other fallow site, Denny Lodge, for nitrogen. Moreover, for magnesium and phosphorus the two roe sites were placed inbetween the fallow sites with respect to their mean content (see Figures 4.13 for magnesium and 4.19 for phosphorus). The two roe sites were only significantly different from one another in the case of calcium, where Squabb vegetation contained relatively very high amounts of this nutrient.

4.4 DISCUSSION

4.4.1 Trends in the quality of vegetation at each site

This analysis considered all the plant species, before composite groups were made, it investigated general seasonal patterns which can be compared to the published literature.

The nitrogen and phosphorus content of plants were the only nutrients that showed seasonal variation and only at the fallow deer sites (Brinken and Denny Lodge), however Figures 4.1 to 4.4 suggest some trends were evident for all the nutrients. With the exception of calcium the majority of nutrients peaked in MarApr and MayJun at all sites (spring peak). This high concentration in the spring coincides with a peak in new growth for most plants (Dietz *et al*, 1958, 1962; Short *et al*, 1966; Ekins, 1989). As the plants aged through the summer months and into the autumn and winter the nutrient content generally declined at each of the sites, although at the roe sites (Great Covert and Squabb) this decline was less marked. The calcium content over the year was very different to the other nutrients at each site. Although not significant, the apparent trend was for the calcium content to increase through autumn and into winter.

In order to compare the current data with published work, it was necessary to convert the bimonth data to seasonal data: spring = March and May (mean), summer = July, autumn = September and November (mean), winter = January. These data are displayed in Table A9.1-A9.5 in Appendix 9 and are compared with data from previous research. It is evident that the data collected from plants at the four sites are similar to those of other researchers suggesting that the techniques used in the current study were accurate and reliable, allowing direct comparison with the literature. There is some debate about the accuracy of phosphorus which will be discussed in Section 4.4.2 in more detail. Calcium occurred in low amounts but was in keeping with Ekins' (1989) findings.

The spring peak suggested earlier is more evident from these tables (Appendix 9). Where other researchers have measured content in the spring, these too are the highest values over the year except in the case of calcium. Most of the study sites (Brinken: nitrogen, magnesium, potassium, phosphorus; Denny Lodge: nitrogen, magnesium, phosphorus; Great Covert: nitrogen, phosphorus; Squabb: nitrogen) also display highest concentrations in the spring, some sites (Denny Lodge: potassium; Great Covert: magnesium, potassium; Squabb: magnesium, potassium) show a peak in the summer this may be due to the makeup of the seasons and the spring data being mean values.

However, Short *et al* (1966) did note seasonal highs in the summer for magnesium. Ekins (1989) divided her year into two seasons to obtain mean values (Appendix 9, Table A9.1). Her summertime period (May-Oct) also covered late spring and she found vegetation contained more nitrogen, potassium and phosphorus around this time as compared to her winter period (Nov-Apr).

Dietz *et al* (1958) found lowest mean **nitrogen** content in the autumn months with values remaining low in winter, this trend was also the case at Brinken (Appendix 9, Table A9.1). The remaining sites showed a drop off in the autumn but the lowest values were in the winter, as noted by Short *et al* (1966) and Ekins (1989).

The only study to investigate more than one season for **magnesium** content was Ekins (1989). She found that the range of concentrations in summer was not dissimilar to that in the winter. This similarity across the year was also found at all four study sites. Although the concentrations of magnesium were low (they do not fall into the upper ranges of Ekins' data [1989]), there was very little difference across the seasons at all four sites. The reason for this is not clear.

The lowest **potassium** concentrations were in the winter for both roe sites and Denny Lodge, this was also the case for most of the other studies (Short *et al*, 1966; Torgerson and Pfander, 1971; Ekins 1989).

Several researchers have looked at **phosphorus** content of vegetation over the seasons (Dietz *et al*, 1958; Short *et al*, 1966; Torgerson and Pfander, 1971; Ekins, 1989) and all except Dietz *et al* (1958) noted a decline in the winter. At the current study sites the lowest phosphorus content was recorded at different times, for Brinken it was in the summer (see Grasman and Hellgren, 1993), for Great Covert it was winter and for Denny Lodge and Squabb it was autumn (see Dietz *et al,* 1958).

The changes between spring/summer and autumn/winter for most nutrients is due to the different growth stages of plants (Dietz *et al*, 1958, 1962; Short *et al*, 1966).

Calcium content of vegetation was very different to the other nutrients. The highest values were in autumn for Brinken, Great Covert and Squabb, and in winter for Denny Lodge. Dietz *et al* (1958) compared all the seasons and found an increase in spring as for the other nutrients. However, Ekins (1989) found the increase in calcium occurred in the winter (November-April) in accordance with the current study, again this may be due to the locality of her study sites. She states that calcium increases with senescence hence the increase content in the latter part of the year. Short *et al* (1966) also comments that calcium is located in the leaves and is present more in old leaves than young ones. Therefore the calcium trends seen at all four sites were to be expected.

Unfortunately many of the studies that have looked specifically at the nutrient content of plants have concentrated on only one season making direct comparisons very difficult. Other researchers show low values for winter (Appendix 9, Tables A9.1 - A9.2) but without comparative spring or winter data, these cannot be conclusive.

4.4.2 Trends in the quality of important forage species

4.4.2.1 Interspecific comparison

The highest mean nitrogen values were for plant species that have complete new growth each year, so that every spring they are full of nutrients including nitrogen (Dietz *et al*, 1958, 1962; Ekins, 1989). These were deciduous trees (Birch and Oak), but also Bramble (N.B. Bramble retain some leaves over the winter period). Conversely, species that had the lowest mean content were conifers (Western hemlock, Douglas fir and Holly) that keep their leaves all year round. These trees do have new growth in the spring, but not to the same extent as deciduous trees which replace all their leaves.

Birch also had the highest mean concentration of phosphorus and Bramble had the highest magnesium, as noted by Ekins (1989). The reason for this may be the same as for nitrogen, increased growth in the spring, however Pine was also included as one of the species containing the highest amounts of phosphorus. Ekins (1989) reports Pine as being of low nutritional quality compared to other evergreens such as Holly. The reason for Holly having a relatively high concentration of phosphorus, in the current study, is uncertain, this adds to the doubts about the measurement techniques (see later). The lowest mean concentrations were for *Polytrichum* spp., Bracken and Douglas fir, suggesting the distinction between deciduous and coniferous was not the main reason for the difference in concentrations.

In general however, plant species with prominent annual growth cycles tended to have high levels of nutrients (nitrogen, magnesium, potassium and phosphorus) in the growing phase (spring) that greatly influenced the mean content. Conifers tended to have more consistent nutrient content over the year hence the means were lower in comparison. Calcium increases with maturity (Ekins, 1989) hence evergreen plant species had more calcium and which then increased the mean value, this was observed for Ivy and Holly, although conifers contained slightly less than Oak and Bramble.

Polytrichum spp. was the species with the lowest mean content of calcium, magnesium and potassium, clearly a very nutrient-poor plant. This was confirmed by Putman *et al* (1981) and Ekins (1989), who found it to be very indigestible. *Polytrichum* spp. together with all the conifers had the lowest amounts of potassium and these were contrasted against the monocotyledons *Carex* spp. and *Juncus* spp. In Ekins' (1989) study of the New Forest *Juncus* spp. had one of the highest concentrations of potassium, 3.85% in April (superceded only by Bracken in May), in the current study *Juncus* spp. contained the highest levels of potassium, 4.07% and 3.06% in MayJun and JulAug respectively.

4.4.2.2 Seasonal variation

Nitrogen and potassium varied significantly throughout the year such that the highest values were in MayJun/JulAug (spring/summer) and the lowest were in JanFeb (winter). This was most likely as a result of the new growth in the spring which continues, or starts in some species, in the summer. Ekins (1989) found that nitrogen, potassium and phosphorus increased during the growing season and then declined as the plants matured. The pattern was reversed for calcium content, which was also significantly affected by bimonth, here the highest mean concentration was in the winter (NovDec) and the lowest in MayJun. As mentioned in Section 4.4.1, calcium increases with plant maturity (Ekins, 1989) thus higher amounts are found in older plants in the winter than younger plants in the spring (Short *et al*, 1966).

The plant species*bimonth interaction produced significant effects for nitrogen, calcium, magnesium and potassium, suggesting a variation in content of different species over the year. Many species differed in the amounts of nutrients they contained at each bimonth, *e.g. Juncus* spp. had low amounts of magnesium in MarApr, Douglas fir in SepOct and *Polytrichum* spp. had low concentrations of every nutrient in most bimonths.

Most species, particularly Oak, Birch and *Juncus* spp., had peak nitrogen and phosphorus contents in spring (MarApr, MayJun) and then declined. Deciduous plants such as Birch, Oak and to some extent *Juncus* spp. have high amounts of nutrients associated with active growth that are withdrawn prior to leaf fall (Ekins, 1989). Potassium peaked slightly later than this for most species (*e.g.*

Bramble, Ivy, *Carex* spp., *Juncus* spp.) in MayJun/JulAug. Magnesium also peaked later in JulAug particularly in Bramble and Birch. In deciduous plants, magnesium is largely retained after the leaves have fallen and so for many species high concentrations were recorded in the winter bimonths, for others there was no decrease and the magnesium content was fairly constant over the year. Calcium did not appear to have a set peak time but it was clearly not between MarApr and JulAug like the other nutrients, the highest times were, for most plants, over the winter period (NovDec - JanFeb). As for magnesium, in deciduous plants calcium is held back in the leaves after they have fallen, therefore higher levels are recorded at this time.

Comparison with literature and reliability of chemical analyses methodologies.

Nitrogen: On comparing the seasonal variation of nitrogen content of the twelve plant species with the work of Ekins (1989; Appendix 8), she found the same seasonal trends in Bramble, Oak and *Juncus* spp., all of which peaked in their nitrogen content in MayJun. Pine contained low amounts of nitrogen, as confirmed by Ekins (1989), and at the current sites there was no difference over the year. Differences between Ekins' data and the present findings were principally for Holly, where she found a peak in June indicative of a late growing season, whereas the current study found Holly contained low amounts of nitrogen with no variation over the year. The reason for this inconsistency is unclear, Holly is an evergreen and as such is more likely to show less variation in its nitrogen content over the year as new growth does not have the same impact as in deciduous plants. Another slight discrepancy was for Bracken, the peak in Ekins' data (1989) was a month earlier (in April) than the current data, this may simply have been to differences in the microclimate of the study years. Most of the other species were not analysed by Ekins (1989) but here showed low concentrations of nitrogen; Ivy, *Polytrichum* spp., Douglas fir and Western hemlock. The remainder, Birch and *Carex* spp., both showed peaks in MayJun.

Most of the current study data therefore coincided with that of Ekins' (1989) therefore the method of nitrogen analysis used and the data produced were considered reliable.

Calcium: Appendix 8 displays all the comparisons for calcium. As for nitrogen, Bramble and *Juncus* spp. produced the same results as those of Ekins (1989). Bramble showed a peak in November in both studies, however Ekins (1989) saw an additional peak in March, the reason for which is uncertain. *Juncus* spp. contained very low amounts of calcium but peaked in SepOct, also shown by Ekins (1989). Calcium content of *Polytrichum* spp. was also the same in both studies; very low, and in the current study there was no difference over the year. Differences between the two studies were again found for Holly, with peaks occurring in JanFeb for the current study and autumn/winter in Ekins' study. The calcium concentration of Oak in the present study peaked in NovDec but in September/October and again in January in Ekins' work. Bracken revealed a peak calcium content in MarApr whereas this was the period of lowest concentration for Ekins, the decrease in the current study was observed later in MayJun and JulAug.

Overall the similarities between Ekins' (1989) data and the current data suggest that the analysis technique for calcium was reliable. There are no obvious reasons for the discrepancies other than slight differences in the growing seasons due to climate or soil chemistry. The latter is thought to be unlikely as the studies were done in very similar localities.

Of the species that could not be compared to Ekins' findings, Ivy peaked in calcium content in JanFeb/MarApr, Birch in SepOct/NovDec, Douglas fir in MarApr and Western hemlock peaked in JanFeb. *Carex* spp. had very low levels of calcium, the lowest were in SepOct.

Magnesium: Of the species Ekins (1989) also analysed for magnesium (Appendix 8) only *Polytrichum* spp. showed similar levels: very low amounts. Bramble and Oak in the current study had their highest concentration of magnesium later than witnessed by Ekins: Bramble in JulAug as opposed to March; Oak in JulAug as opposed to May. The peak concentration of magnesium over the year varied between plants, for Holly it was MarApr and again in JulAug; for Ivy it was SepOct; for Birch JulAug (as Oak); for Bracken NovDec/JanFeb; for Pine MayJun; for Douglas fir MarApr. At any one time of year one species will contain high levels of magnesium. Ekins' data (1989) described an increase in the magnesium content of Holly in autumn/winter and low concentrations in Pine. She also described a steady increase in magnesium content for *Juncus* spp. peaking in October, whereas the current study showed low concentrations all year round. *Carex* spp. also contained low levels and Western hemlock showed no difference in concentration over the year.

Although the results from the current study do not match those found by Ekins (1989) the ranges of magnesium content for the studies were similar (Appendix 9, Table A9.3), therefore allowing for the different seasonal variations in the two studies, the method used for recording the magnesium content of plant species was considered valid and accurate.

Potassium: This nutrient displayed the greatest similarity between this and Ekins' (1989) studies (Appendix 8). The species in agreement were Bramble, the potassium content peaked in MayJun; Oak also showed a peak in MayJun (the current study also showed a second peak in SepOct); *Juncus* spp. peaked in MayJun/JulAug; Pine and *Polytrichum* spp. contained low amounts of potassium. Holly showed no difference over the year whereas Ekins' data (1989) produced a peak in June. Bracken peaked later in the current study than in Ekins', MayJun/JulAug in contrast to April. Ivy and *Carex* spp. both had peak concentrations of potassium in JulAug. In agreement with Pine, the other conifers analysed (Douglas fir and Western hemlock) also had very low concentrations of potassium.

The method of analysis used to determine potassium content in the plant species was regarded as reliable as the majority of measures from both studies coincided.

Phosphorus: Similarities were found (Appendix 8) between the current study and Ekins' data (1989) with regard to phosphorus content for Oak and *Juncus* spp., both peaked in MayJun. Bracken also peaked in MayJun, slightly later than Ekins' data (1989). Bramble likewise, peaked slightly later in JulAug in contrast to May/June. The current data showed Pine had the highest concentrations of phosphorus in MayJun and again in SepOct, whereas Ekins described this conifer as having low amounts of phosphorus. Douglas fir contained low amounts but did have a peak in MayJun, Western hemlock had an earlier peak in MarApr. Holly, as for nitrogen and potassium, showed no difference over the year in contrast to a peak in June shown by Ekins. Ivy had a peak phosphorus content in JulAug and *Polytrichum* spp. had very low levels as was the case for all the nutrients analysed.

The degree of similarity in seasonal patterns of phosphorus content between the two studies suggests that the analysis technique was reliable and that the results were valid. However the actual range of phosphorus content suggests that there may have been some irregularities in the analysis. Table A9.5 in Appendix 9 displays the seasonal amounts of phosphorus found. The winter values of the current study are feasible (especially with Ekins' [1989] data), but the other seasons appear to contain very low amounts when compared to Ekins' and other studies. The reasons for this are unclear and question the reliability of the phosphorus data. Future relationships with phosphorus should therefore be considered with caution.

4.4.2.3 Inter-site comparisons

The reason why the plants at Brinken were relatively nutrient poor is unclear, it could be due to the geology of the sites or differing management practices. Ekins (1989) investigated the relationship between plant quality and soil chemistry. Unfortunately she did not have reliable data for nitrogen and phosphorus, but found that the calcium and magnesium contents of the soil were positively correlated with those of the plants. Potassium showed no correlation. The soils at Brinken and Denny Lodge are acidic, as is much of the New Forest (Tubbs, 1986) and vegetation on such soils are known to contain less calcium than better soils (Kincaid, 1988), this may suggest why Brinken was so nutrient poor, particularly for calcium.

The significant interaction throughout between plant species and site indicates that the different plants have different nutrient profiles at each site. This emphasises the need to consider each site as a separate entity, especially when comparing the nutrient content of a plant species in relation to how much of it was eaten (Chapter 5).

4.4.3 Digestibility

These values must be considered separately as they are from published data (Putman *et al*, 1981) and their main reason for incorporation is in the interpretation of diet selection in Chapter 5. However, seasonal trends and differences between the plants can be highlighted. Ivy was the most digestible plant (mean = 72% dry matter digestibility) and this remained the case for the entire year. Holly was also very digestible for most of the year except for the summer months. Oak showed an increase in digestibility with the growing season which then declined as the proportion of mature and senescent material increased (Ekins, 1989). Bramble was highly digestible in the winter months but was much lower the rest of the year. The remaining plant species were relatively low in digestibility, Bracken, Moss and *Juncus* spp. were the lowest for most of the year.

The mean digestibility of the plant species important in the diet showed very little difference over the year, unlike the findings of Torgerson and Pfander (1971) and Drodz (1979) who found differences between summer and winter. The lack of variation in the Putman *et al* (1981) study was due to the similarity of digestibility measures within each species, *e.g.* Ivy was highly digestible throughout the year (range: 62-84%) and *Juncus* spp. was consistently low (range: 22-35%).

4.4.4 Summary and general discussion

The most important finding for the main aim of this study, *i.e.* diet selection based on nutrient content, was that seasonal variation was shown in all nutrients. Although only significant for nitrogen and phosphorus when using all the nutrient data, similar trends were suggested for the other nutrients and were confirmed when the important forage species only were considered.

The greatest seasonal variations in forage quality were observed in plants with distinct annual growth cycles. Leaves of deciduous species such as Oak and Birch were highly nutritious when young. Withdrawal and leaching of nutrients as the plants mature coupled with increases in fibre render the plants largely indigestible and unpalatable (Ekins, 1989). The relationship between the quality of vegetation eaten and the amounts in which it was eaten over the year will be investigated in the following chapter. This will attempt to answer the question "did the nutrient composition of plant species influence how much was eaten at different times of the year?" This is diet selection in its most simplistic form and will have to consider availability (Chapter 3) as well as factors such as toxins, physical attributes etc.

CHAPTER 5 DIET SELECTION IN THE NATURAL ENVIRONMENT

ABSTRACT

Data on the composition of fallow and roe deer diets were calibrated against diet quality, represented by nutrient content. Data were collected over a 12 month period, subsequently reduced to six bimonths (two month blocks), at four sites, two with fallow deer only (Brinken and Denny Lodge) and two with roe deer only (Great Covert and Squabb). Diet selection based upon nutrient content was estimated from correlations and from triangular diagrams for the three most commonly eaten plant species, and were related to published nutrient requirements. Results from the correlations suggested that both fallow and roe deer were selecting for and against certain plant species based upon their nutrient content but that availability was also involved. Comparing the nutrient intake of the three most commonly eaten plant species with known requirements showed that in winter particularly these plant species, could provide sufficient nutrients for maintenance levels to be achieved. However, when the females were in stages of late pregnancy and subsequent lactation most did not achieve their protein and calcium requirements even though in some cases the vegetation could provide the required levels. Male fallow deer took a diet that was deficient in calcium during regrowth of their antlers, however this did not appear to be case for roe deer, which have relatively smaller antlers growing at a different time of year. It was assumed that both species of deer, particularly the females, rely heavily on their nutrient reserves at certain times of year.

5.1 INTRODUCTION

This chapter considers diet selection by fallow and roe deer in their natural environment. This will be investigated at each site by combining the quantitative data from the diet composition (Chapter 2) with the qualitative data on the nutrient makeup of each important plant species in the diet (Chapter 4) and by considering the availability (Chapter 3). The aim is to uncover trends between nutrient content and the amounts eaten of specific plants and answer the main question, **"do fallow and roe deer select specific foods based upon the nutrient content?"**

Providing evidence for the presence or absence of diet selection can be very difficult. The theory can be extremely complex as discussed in Section 1.3. This chapter aims to investigate diet selection by asking two separate questions:-

1) **"are fallow and roe deer getting sufficient nutrients to meet their known requirements?"** This will be addressed by looking at the diets the deer selected in terms of nutrient concentrations and relating these to published accounts of requirements.

2) **"are fallow and roe deer selecting plants according to their nutrient content?"** This will be addressed by correlating nutrient concentration and proportion present in the diet over the year for all important forage species individually.

Both of these have inherent problems but the main concern for the first investigation are the actual nutrient requirements for the deer. Very little work has looked specifically at the requirements for fallow and roe deer. Most of the research into requirements has been done for white-tailed deer and mainly for protein (French *et al*, 1956; McEwan *et al*, 1957; Dietz, 1965; Murphy and Coates, 1966; Ullrey *et al*, 1967; Smith *et al*, 1975; Holter *et al*, 1977, 1979). Many authors have looked at the overall needs at certain ages, *e.g.* for maximum growth in fawns (Ullrey *et al*, 1967; Smith *et al*, 1975) or for antler development (McEwan *et al*, 1957). There is only one known published account of protein requirements for fallow deer (Reinken *et al,* 1990) and one of red deer (Adam, 1988), both written for deer farmers. In this chapter, reference is made to the published requirements of white-tailed deer but the data of Adam (1988) and Reinken *et al* (1990) are used in preference, particularly as these data are given for different times of the year. There is even less information for nutrients other than protein. Reinken *et al* (1990) list the levels of calcium, magnesium and phosphorus required by fallow deer in the winter. Adam (1988) uses data extrapolated from domestic ruminants for the same nutrients and also potassium. Several authors have listed the ideal calcium:phosphorus (Ca:P) ratios for white-tailed deer (French *et al*, 1956; Dietz *et al* 1958; Dietz, 1965).

The lack of data for roe deer dictated that their results be discussed in terms of fallow and red deer requirements. This is not very satisfactory given that roe are very different kinds of feeders to these species of deer (Section 1.2.2), however there was no alternative. Where possible the data from the roe deer is discussed outside of the published requirements.

Another major consideration in diet selection is the digestibility of forage species. The nutritive value of a plant species refers not only to the chemical composition, but also the digestibility of that species (Barnes, 1965 cited in Drodz, 1979). Many early authors described the quality or nutritive value of an animal's diet by simply referring to the chemical or nutrient makeup (*e.g.* Hellmer, 1940). More recently researchers have determined the digestibility of the main plant species in the natural diet (*e.g.* roe deer: Drodz, 1979) to gain an insight into nutritive value. True digestibility can only be gained from controlled experimental trials. Specific nutrients are measured on intake and again in the faeces produced, from this the amount of digestible nutrient can be calculated (*e.g.* Dietz *et al*, 1962; Papageorgiou *et al*, 1981). For example, the digestible crude protein of plants can be calculated instead of the actual crude protein content. There is still much discussion about measuring digestibility, many factors that affect it can not always be measured (*e.g.* methane, see McDonald *et al*, 1981).

The nature of the current study dictated that deer could not be penned then fed important forage every bimonth measuring nutrient input and output and thereby digestibility. Digestibilities of all important forage species over the year were used (Section 4.3.5.6) having been measured *in vitro* by Putman *et al* (1981) as described in Ekins (1989). The nutrient content of each plant species was then considered in view of its digestibility for that particular bimonth. Drodz (1979) regarded that a mean digestibility of 70% indicated that all the nutrients would be accessible to the deer. In the study by Putman *et al* (1981) only Holly in February was >70% digested, therefore for the current study a digestibility of 50% or more was considered to indicate that most of the nutrients were available to the deer. As no digestibility coefficients (measured from input and output) were calculated in this study, the nutrient data will be considered with diet composition as it was measured, *i.e.* without incorporating digestibility (Torgerson and Pfander, 1971). The digestibility measures will be used to put the findings into context in the discussion (Section 5.4).

The effects of secondary plant metabolites (toxins, *e.g.* tannins) will not been addressed in this chapter primarily because they were not measured. Tannins are presumed to adversely affect nutrient content, however certain tannin producing plant species are nutritious and are readily ingested (Robbins *et al*, 1987a). Different tannins have different effects on mammalian herbivores, some animals are more tolerant than others (Provenza *et al*, 1990), roe deer are relatively insensitive, eating foods with high tannin concentrations (Duncan *et al*, 1998). Other secondary plant metabolites such as monoterpene hydrocarbons in Douglas fir needles have only slight or no effect on deer rumen microbial activity (Hi Kon Oh *et al*, 1967). Therefore, although it is generally assumed that plant toxins will be avoided by herbivores this is clearly not always the case or indeed necessary. An overall generalisation about the effects of toxins when interpreting the results of the current study will therefore not possible. The data could only be considered in terms of the macronutrients measured.

Data from the proportion of plant species eaten in the diet (Section 2.3.4) and the nutrient content of those species (Section 4.3.5) were combined. The data were analysed using two very different methods thus the methodologies and results sections are each described separately for clarity. Firstly the nutrient content of plant species eaten in the greatest volumes were examined (Sections 5.2) and secondly, a correlation was performed between the majority of the plant species eaten and their nutrient content (Sections 5.3). There are advantages and disadvantages for both methods and the limitations will be considered jointly in the discussion (Section 5.4.4).

5.2 MOST ABUNDANT SPECIES EATEN AND THEIR NUTRIENT CONTENT

5.2.1 Methodology

Triangular diagrams, generally used for data with three components (*e.g.* classification of soils), were adopted and modified for this study. Vulink and Drost (1991), investigating diet composition of free-ranging cattle, used these diagrams and combined the species eaten into three classes; grasses, reeds and forbs. These classes were then used to create diagrams and display the data at different times of the year. Levels of different nutrients were also plotted on to the diagrams.

Of interest in the current study were the quantities of individual plant species eaten and their nutritional content. The three most commonly eaten plant species or groups in a bimonth were used to create the diagrams (Appendix 10). Where plant groups were used the mean nutrient content had been shown to be reliable if calculated for each site separately (Section 4.3.3). At Great Covert the Herb and Monocotyledon groups were jointly the most commonly eaten groups in MayJun and JulAug. The latter group was used as the mean calcium content of the Herb group had previously been found to be unreliable (Section 4.3.3.1).

In order to use these diagrams the amounts of the three plant species in each bimonth had to be recalculated to add up to 100%, as the deer had eaten more than three plant species. Data for each individual deer (*i.e.* each original faecal sample see Section 2.2.2) were used from each month and were recalculated using the example in Table 5.1. This provided up to 20 data points on each diagram for each bimonth (see Figure 5.2a, as an example).

Table 5.1 Example of the recalculation: three plant species eaten by Deer 1 in January

Plant species	Proportion of diet	Recalculation	Proportions used for diagram
Holly	40%	(40/40+23+25) x 100	46%
Ivy	23%	(23/40+23+25) x 100	26%
Moss	25%	(25/40+23+25) x 100	28%

The requirement levels of deer for all the nutrients (Table 5.2) were taken from the most comprehensive published information on the nutrient requirements of deer (red deer: Adam, 1988; fallow deer: Reinken *et al*, 1990).

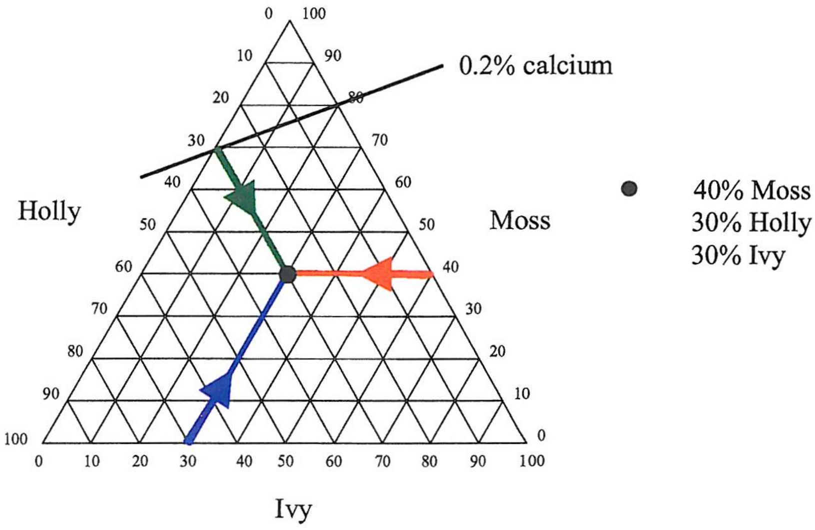
Table 5.2 Published nutrient requirements used to determine requirement levels for triangular diagrams

	Adam (1988) %	Reinken *et al* (1990) % (winter data only)	Requirement levels used %
Protein	10 - 17	6 - 11	6, 10, 12, 14, 17
Calcium	0.3 - 0.6	0.3 - 0.5	0.3, 0.6
Magnesium	0.1 - 0.2	0.04 - 0.05	0.1, 0.2
Potassium	0.3		
Phosphorus	0.2 - 0.4	0.2 - 0.3	

Figure 5.1 shows an example of how the triangular diagrams work. The lines from each point on each axis leave at the same angle but because of the orientation of each axis the observed grid is created. The coloured arrows show the direction of the line from each axis. In this example Holly, Moss and Ivy were the three most commonly eaten plant species for this bimonth and the point plotted is for a deer that ate 30% Holly, 40% Moss and 30% Ivy (recalculated). Using the chemical content data for specific plants supplied in Chapter 4, the requirement line for 0.2% calcium has also been plotted, this was deduced as 80% of the calcium content of Moss plus 20% of the calcium content of Ivy produce 0.2% calcium, as does 70% of the calcium content of Moss plus 30% of the calcium content of Holly. Any point along this line will give 0.2% calcium, the end points of the line use only two plant species, anywhere in between uses all three. A point plotted (a deer) above this line indicates that a diet containing more than 0.2% calcium was eaten.

This was done for each nutrient and the findings interpreted (Section 5.2.2). Nitrogen content was converted to crude protein content (%CP) using an agreed constant (see Appendix 6). The calcium: phosphorus ratio was also investigated.

Figure 5.1 Example of a triangular diagram: coloured arrows indicate the orientation of each axis. A point (deer) and the requirement line (0.2% calcium) have been plotted to show how the diagram works.



5.2.2 Results

The results from the triangular diagrams will be compared with published nutrient requirements for deer. As the sex and age of each deer could not be determined in this study, each point (deer) on the graphs (see Figure 5.1) will be considered as potentially male or female and adult (1+years), when being discussed.

5.2.2.1 Protein (nitrogen)

Table 5.3 shows the protein requirements of red deer (Adam, 1988) and fallow deer (Reinken *et al*, 1990). The different physiological states of fallow and roe deer have been incorporated to indicate the increasing needs at certain times of the year and life cycle. All the protein data acquired in this study will be compared with this table.

Fallow deer

January - February

The majority of fallow deer took between 6 and 10%CP (Brinken: 100% of the deer; Denny Lodge: 60%). At Denny Lodge the remaining 40% took just over 10%CP (Figure 5.3a). According to Table 5.3 the requirement at this time of year ranges from 6 to 10%CP for females, and 6 to 11%CP for males. Therefore all the deer in this study were fulfilling their requirement based on the three most commonly eaten plant species.

March - April

All the deer at Brinken took between 10 and 12%CP (maximum = 11.7%CP, Table A10.1, Appendix 10) and most at Denny Lodge took between 12 and 14%CP (Figures 5.2b and 5.3b) in this bimonth. For the females, in the stages of late pregnancy, this falls just below the stated requirement of 14%CP (Table 5.3). For the males, who would be casting their antlers and increasing their body weight, the requirement of 12%CP is within that eaten at Denny Lodge and falls just short of that at Brinken. At Denny Lodge several deer were clustered around 14%CP (Figure 5.3b), therefore any females within this group would have fulfilled their requirement. One animal took in excess of 17%CP, eating mainly Monocotyledons (Figure 5.3b).

May - June

At Brinken just over half the deer took 10-14%CP and the remainder 14-17%CP (Figure 5.2c). At

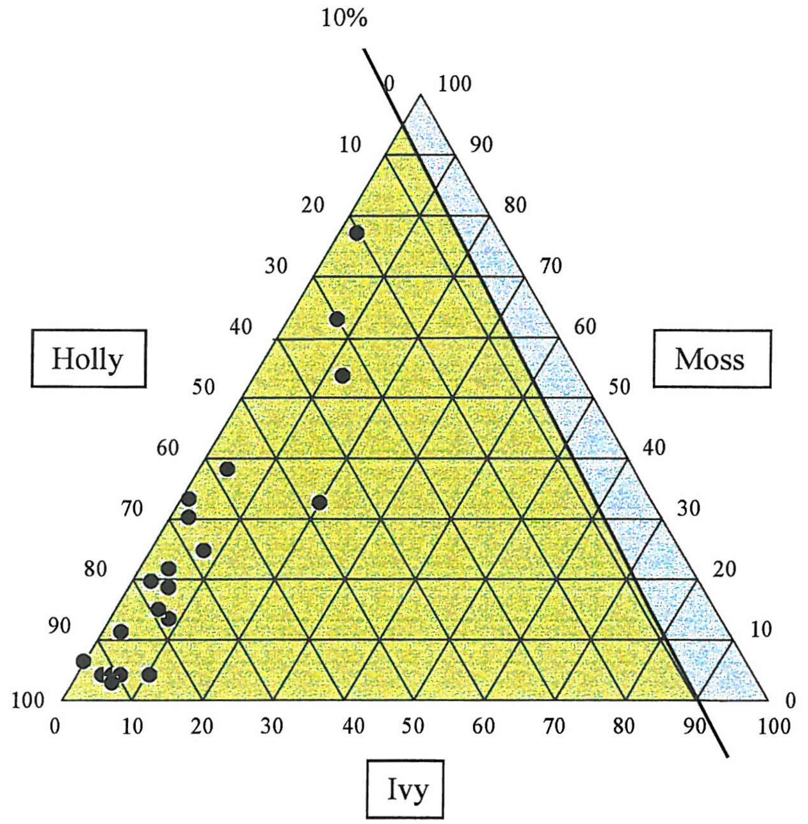
Table 5.3 Protein requirements of red deer and fallow deer during their different physiological stages (physiological stages of roe deer also added)

	Females				**Males**			
	Physiological stage		**Protein requirement (%CP)**		**Physiological stage**		**Protein requirement (%CP)**	
	Fallow and Red deer	***Roe deer***	***Red deer*** [†]	***Fallow deer*** [‡]	***Fallow and Red deer***	***Roe deer***	***Red deer*** [†]	***Fallow deer*** [‡]
JanFeb	mid pregnancy	early - mid pregnancy	10	6 - 8.5	maintenance	antler growth / maintenance	10	6 - 11
MarApr	late pregnancy	late pregnancy	14		increasing body weight / antlers cast	increasing body weight	12	
MayJun	late pregnancy / birth / lactation	birth / lactation	14 - 17		increasing body weight / grow antlers	increasing body weight	12	
JulAug	lactation	lactation / conception (delayed implantation until late Dec early Jan)	17		increasing body weight / grow antlers	rut	12	
SepOct	lactation or dry / conception	lactation / conception	10		rut	maintenance	10	
NovDec	early pregnancy	lactation	10	6 - 8.5	late rut / maintenance	antlers cast / maintenance	10	6 - 11

[†] Adam (1988)

[‡] Reinken *et al* (1990) values for male and female fallow from 1 year to 2/3 years of age.

Figure 5.2a Proportions of the three most commonly eaten plant species at Brinken in JanFeb in relation to their crude protein content (%CP) and with reference levels indicated.



Legend:

● = one deer (sample)

🟨 6-9.9%CP

🟦 10-11.9%CP

This diagram is enlarged as an example of how the data were plotted and how the diagrams work. The following figures have been reduced in size to allow visual comparison between the bimonths.

Figure 5.2 Proportions of the three most commonly eaten plant species at Brinken each bimonth in relation to their crude protein content (%CP) and with reference levels and deer (dots) indicated.

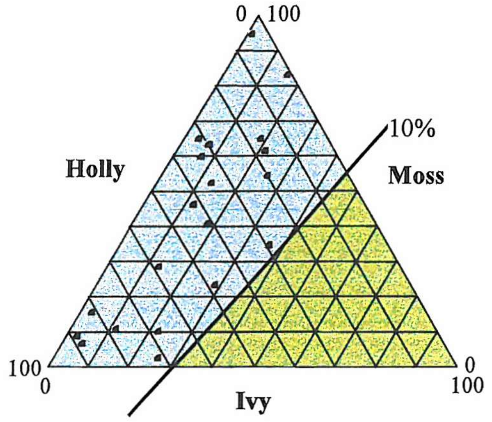
Legend:

🟨 6-9.9%CP ⬜ 10-11.9%CP 🟩 12-13.9%CP 🟦 14-16.9%CP 🟪 >17%CP

a)

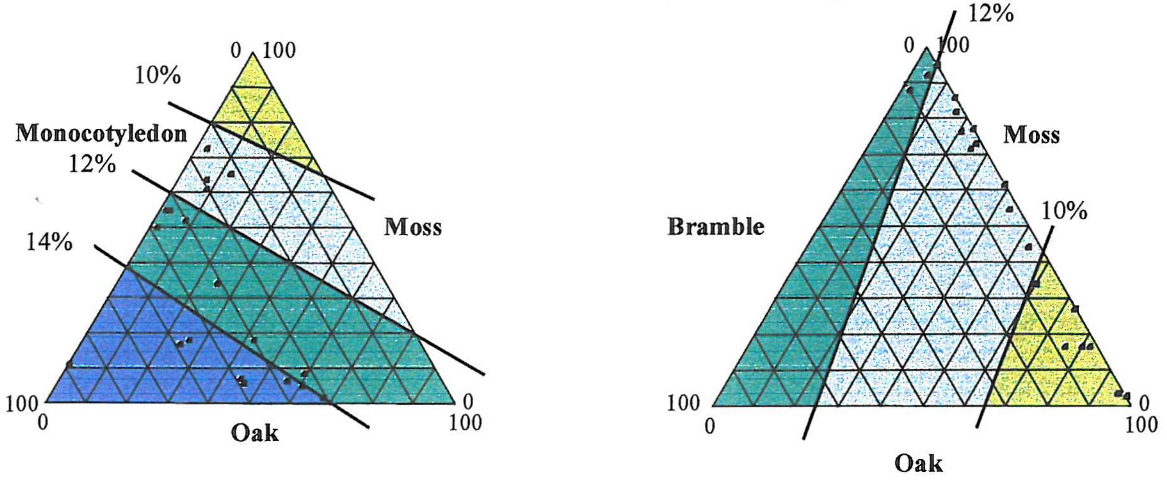
see previous page

b) MarApr



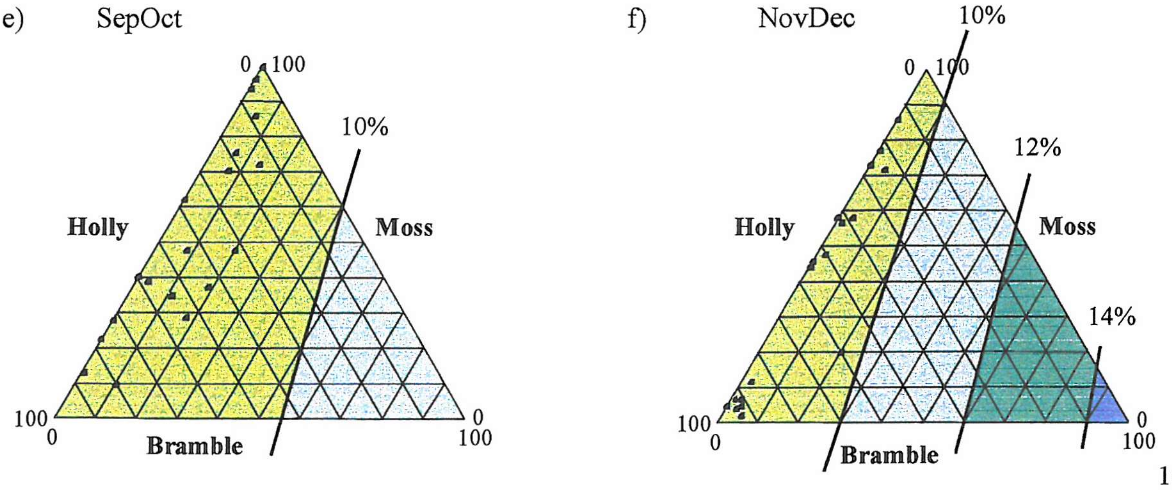
c) MayJun

d) JulAug



e) SepOct

f) NovDec



Denny Lodge most took around 16%CP and some took more than 17%CP (Figure 5.3c).

In MayJun female fallow deer need between 14 and 17%CP in their diet as they are in the stages of late pregnancy, birth and then lactation (Table 5.3), the latter dictating very high protein requirements. At Brinken almost half of the females fulfilled this requirement. All of the females at Denny Lodge took a diet suitable for their requirements during lactation.

Male fallow deer do not need as much protein at this time of year, they are regrowing their antlers and increasing their body weight, and have a requirement of 12%CP (Table 5.3). This level could easily be fulfilled at each site and was the case for most of the deer overall. Of the remainder, four at Brinken took 11-12%CP.

July - August

At Brinken seven deer took less than 10%CP, ten took 10-12%CP and three took 12%CP or slightly more (Figure 5.2d). The most protein rich food only contained a maximum of 13.5%CP (Oak) whereas at Denny Lodge 15%CP (Monocotyledon) was the maximum available (Table A10.1, Appendix 10). At Denny Lodge all except one deer (10-12%CP) took a diet of above 12%CP (Figure 5.3d).

The females at each site would still have been lactating in this bimonth and so their protein %CP requirement would still be high (Table 5.3). At Brinken, even if they ate just Oak they could not have obtained all the protein needed (Table A10.1, Appendix 10). At Denny Lodge they could have got closer to their requirement (17%CP) by eating just Monocotyledons and Coarse grass but many ate these in combination with Moss (Table A10.1, Appendix 10). Compared to the high requirement of the females the males' was still relatively low at 12%CP (Table 5.3) but although this level was attainable at Brinken, most of the deer (85%) took less than this (Figure 5.2d). At Denny Lodge all the deer took in excess of 13%CP so if male they would have easily fulfilled their protein requirements (Figure 5.3d).

September - October

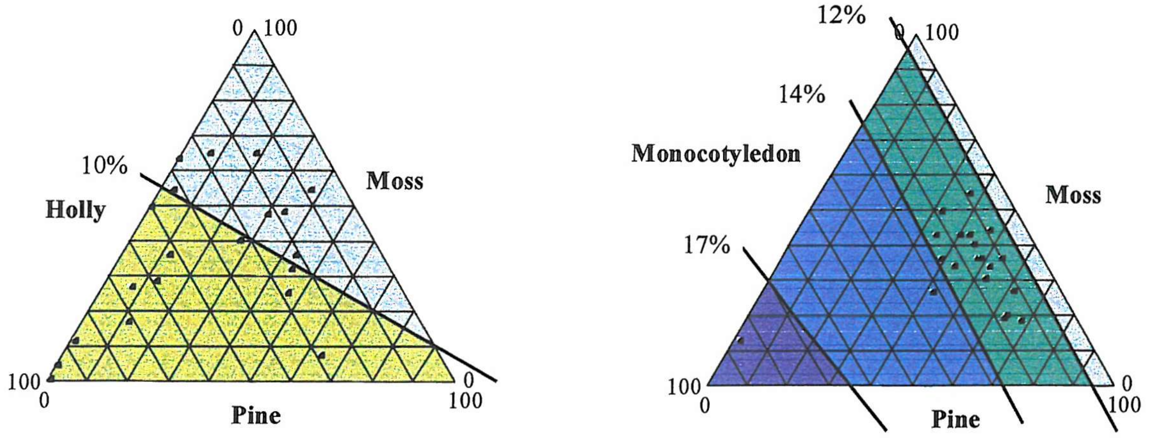
This is the mating season for fallow deer (Table 5.3). The males start rutting and for most of the females lactation is usually tailing off whilst others can already be dry, however lactation has been recorded for up nine months (Chapman and Putman, 1991). As a result protein requirements of both sexes usually decrease at this time of year to just 10%CP. At Brinken, although a diet of 10%CP was available, all of the deer were concentrated in the 6-9%CP range (Figure 5.2e). This may have been

Figure 5.3 Proportions of the three most commonly eaten plant species at Denny Lodge each bimonth in relation to their crude protein content (%CP) and with reference levels and deer (dots) indicated.

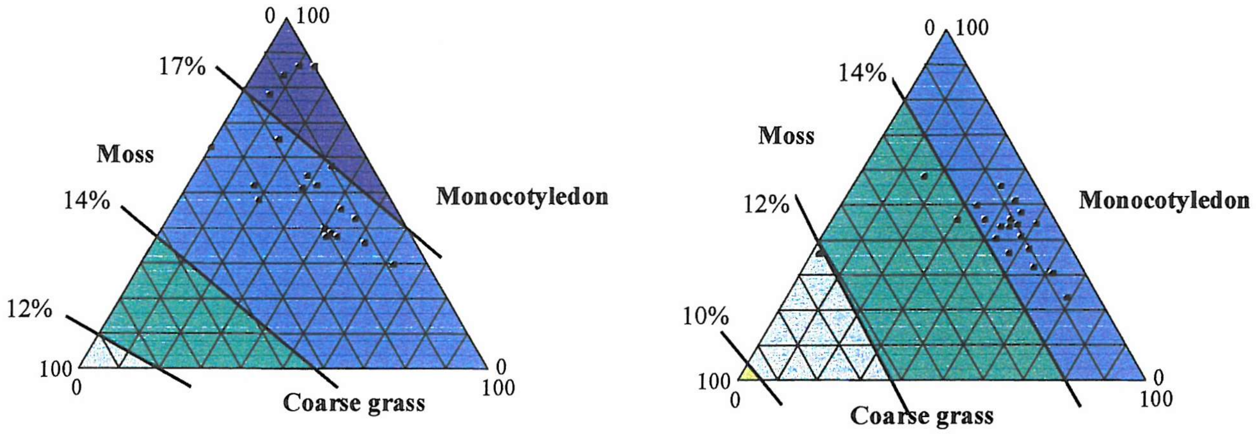
Legend:

🟨 6-9.9%CP ▢ 10-11.9%CP 🟩 12-13.9%CP 🟦 14-16.9%CP 🟪 >17%CP

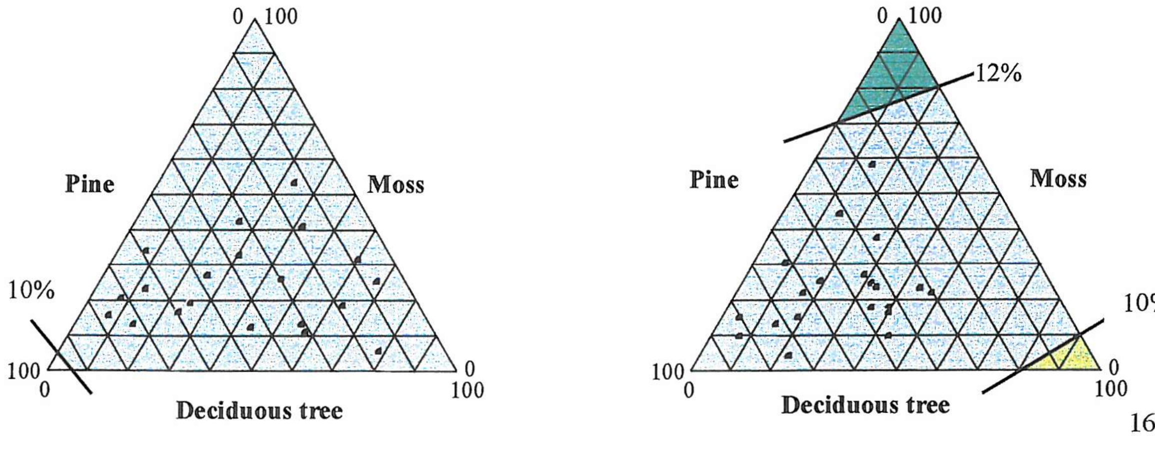
a) JanFeb b) MarApr



c) MayJun d) JulAug



e) SepOct f) NovDec



influenced by other factors such as the ongoing rut dictating where and when the animals can feed. At Denny Lodge, however, all the deer took diets of 10%CP or more (Figure 5.3e), here Deciduous trees featured greatly in their diets and this group had a relatively high protein content (11.6%CP - Table A10.1, Appendix 10).

November - December

At Brinken there was potential to take up to 14.7%CP (all Bramble) but as in the previous bimonth the deer took much less: approximately 9%CP (Figure 5.2f). At Denny Lodge the deer took between 10 and 12%CP (Figure 5.3f). Despite the differences between the two sites, all the deer were within or exceeded the published requirements of 6-10%CP for females and 6-11%CP for males (Table 5.3).

Roe deer

January - February

All the deer at Great Covert took between 6 and 10%CP (Figure 5.4a) during this bimonth, but there was sufficient protein in the three most commonly eaten plant species to provide them with up to 11.2%CP. At Squabb all the deer took between 10 and 12%CP, and the maximum they could take was 12.2%CP (all Pine), although most took a combination of all three plants (Figure 5.5a). The documented requirement at this time of year is 6-10%CP for the females and 6-11%CP for the males, thus the deer at both sites fulfilled their requirements (Table 5.3).

March - April

At Great Covert most of the deer (75%) took a low protein diet of approximately 9%CP relative to that available (maximum = 17.6%CP, all Monocotyledon), the remainder took closer to 13%CP (Figure 5.4b). The majority of deer (84%) at Squabb also took less than 10%CP (Figure 5.5b) but here the maximum available was 13.5%CP (all Ivy - Table A10.1, Appendix 10).

As with fallow deer this bimonth is also the period of late pregnancy for roe deer and as a result females would require 14%CP (Table 5.3). Males require 12%CP for increasing their body weight in preparation for the rut (Table 5.3). At both sites most males and females fell markedly below their requirements.

May - June

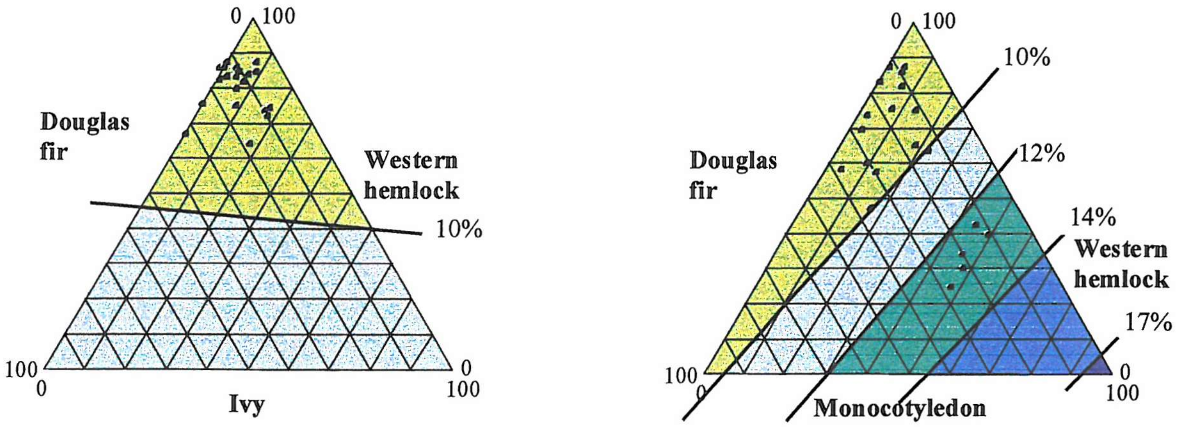
The three plant species eaten most at this time of year at Great Covert yielded a wide spectrum of available protein (6-27%CP). Just under half the deer took less than 14%CP, in contrast 6

Figure 5.4 Proportions of the three most commonly eaten plant species at Great Covert each bimonth in relation to their crude protein content (%CP) and with reference levels and deer (dots) indicated.

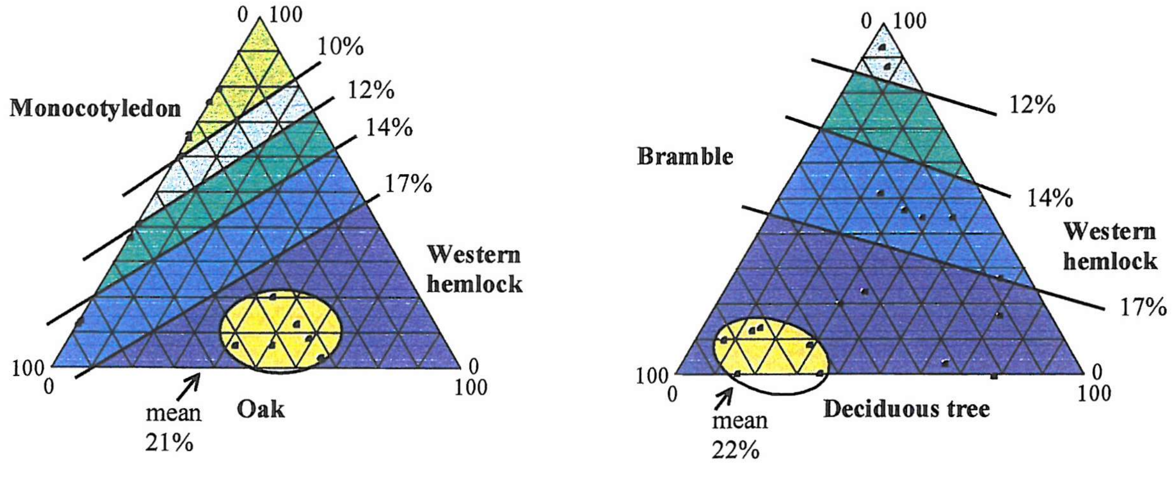
Legend:

▨ 6-9.9%CP ▨ 10-11.9%CP ▨ 12-13.9%CP ▨ 14-16.9%CP ▨ >17%CP

a) JanFeb b) MarApr



c) MayJun d) JulAug



e) SeptOct f) NovDec

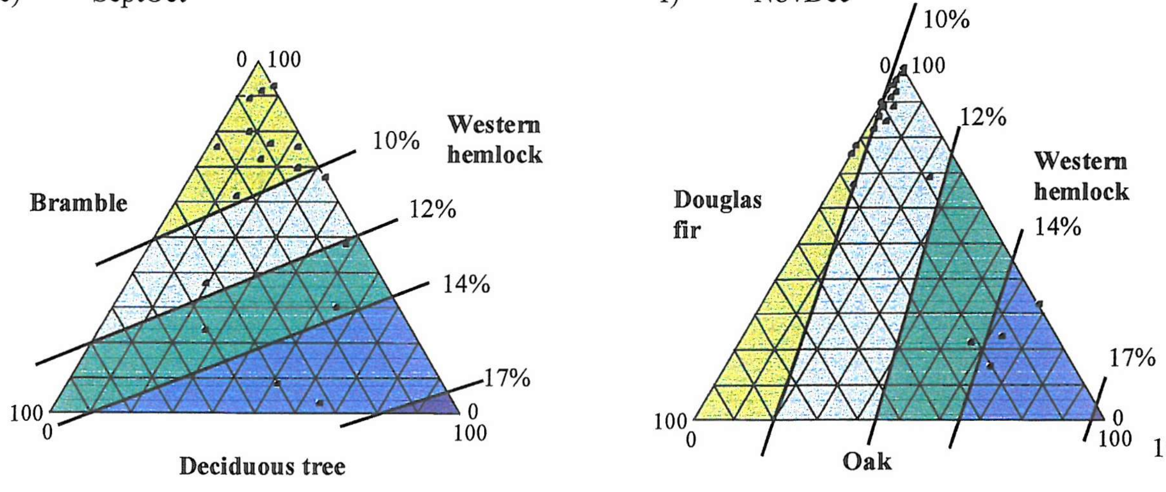
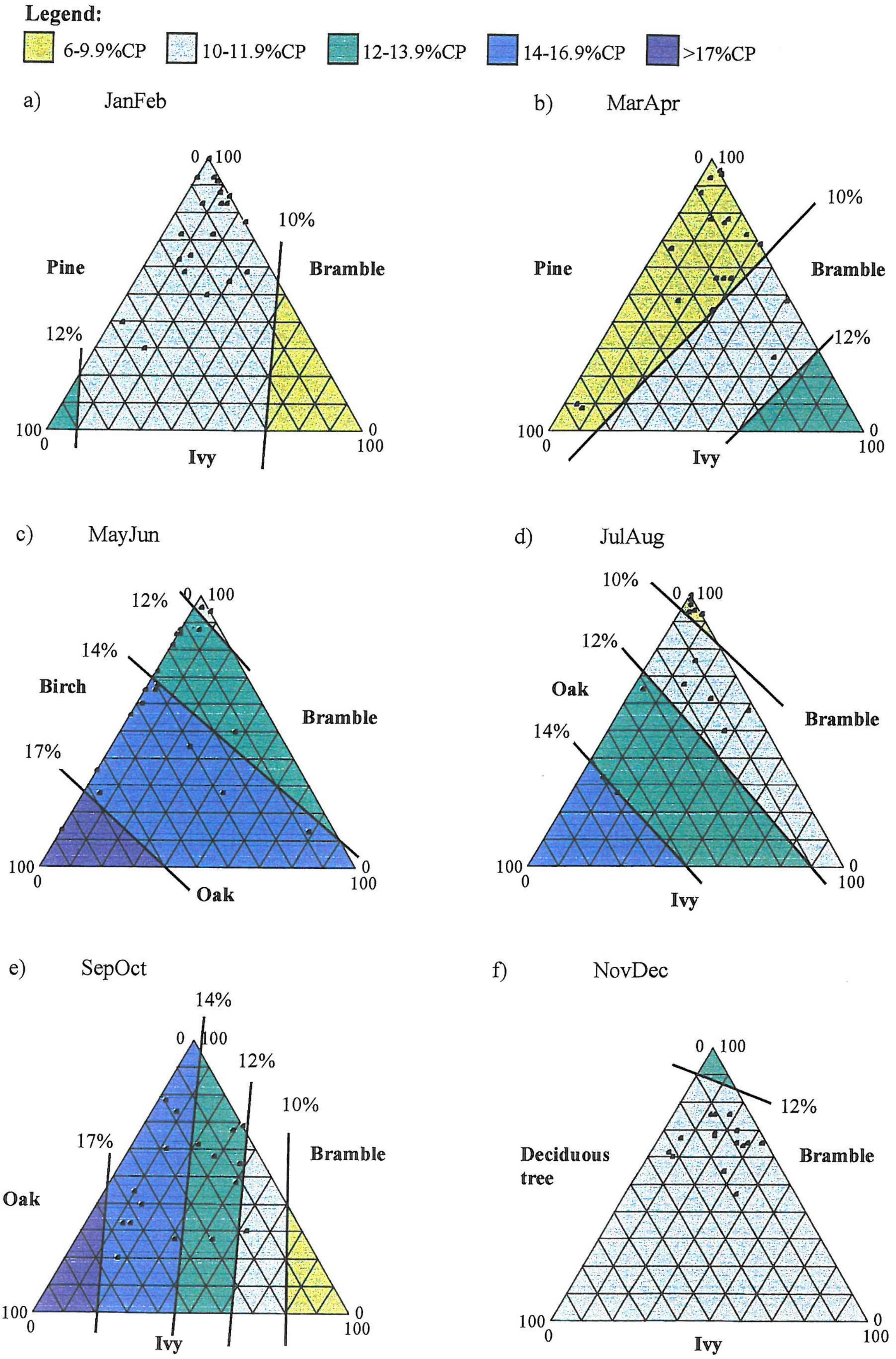


Figure 5.5 Proportions of the three most commonly eaten plant species at Squabb each bimonth in relation to their crude protein content (%CP) and with reference levels and deer (dots) indicated.



individuals took a mean of approximately 21%CP (Figure 5.4c). This wide range of protein intake was due to the presence of Oak in the diet in June which also had the highest protein content (26.8%CP - Table A10.1, Appendix 10) of the three plant species.

Female roe deer give birth during this bimonth and therefore have great demands from lactation. As a result their protein requirement is high 14-17%CP (Table 5.3). At Great Covert these demands were easily covered by the protein content of the plant species eaten, especially in June when many of the deer selected a diet that was more than 17%CP although not all individuals took advantage of the high amounts available. Males need 12%CP at this time of year (Table 5.3) and again the majority of individuals (two thirds) at Great Covert took this level or more.

At Squabb the upper limit provided by the vegetation was not quite as high (19.5%CP - Table A10.1, Appendix 10) as that at Great Covert. The majority of deer (55%) took 14-17%CP, of the remainder 35% took between 12 and 14%CP and 5% took just over 17%CP (Figure 5.5c). All the deer exceeded the male requirements and most fell within the optimum range for females.

July - August

At Great Covert the maximum yielded was 23%CP from Bramble (Table A10.1, Appendix 10). Two individuals took 10-12%CP, four took 14-17%CP, and the remaining 11 took more than 17%CP (Figure 5.4d). Of those taking 17%CP or more, six animals actually took a diet with a mean protein content of approximately 22%CP, a similar upper limit as the previous bimonth.

At Squabb, again there was not the same range as at Great Covert and many of the deer took a lower amount of protein, 43% took less than 10%CP, 35% took 10-12%CP and of the remainder one animal took approximately 12%CP and two others took 14%CP (Figure 5.5d). Individuals that took less than 10%CP had eaten predominately Bramble and little else (Figure 5.5d), which only contained 9.8%CP at this time of year. There was potential for the deer to take up 16.7%CP (maximum = Oak).

The females are still lactating at this time and although they will also conceive next year's offspring, delayed implantation means there should be no extra demands in terms of protein other than for feeding their remaining offspring (Table 5.3). At Great Covert there was potential for selecting a diet that was 17%CP or more (Table A10.1, Appendix 10) and many of the deer took such a diet. At Squabb it was not possible to select a diet that was this rich (maximum = 16.7%CP) and many of these deer actually took a diet less than 12%CP and indeed appeared to select Bramble over anything else.

Male roe deer rut at this time of year and although the reported requirement for red deer is 12%CP in JulAug (Table 5.3), red deer and fallow deer rut later, so a roe male's requirement is likely to be more in line with that of a rutting red deer, 10%CP, particularly as they do not eat much during this time (fallow: Chapman and Chapman, 1975; red: Staines, 1991). Apart from the deer that only ate Bramble (Squabb) which was just under 10%CP, most deer ate more than this level so fulfilled their requirements if they were rutting males.

September - October

At Great Covert 59% of deer took 6-10%CP (the minimum was 6.1%CP - Table A10.2, Appendix 10), 17% took 10-13%CP and the remainder took more than 12%CP with two animals taking approximately 16%CP (Figure 5.5e). The plant species at Squabb yielded higher levels of protein for this bimonth, up to 19.5%CP (Oak). Most of the deer (53%) took a diet between 14 and 17%CP and the majority of the rest took 12-14%CP (Figure 5.5e). Despite there being a chance of taking 17%CP or more (Table A10.1, Appendix 10) none of the deer took this opportunity.

The requirement for both males and females in SepOct is 10%CP (Table 5.3), all the deer at Squabb fulfilled this requirement but few at Great Covert did, with most selecting between 8 and 10%CP.

November - December

More protein was available in this bimonth than the previous one at Great Covert due to the increase of Oak in the diet. As mentioned above, at this time of year this plant species was probably quite unpalatable but was still very high in protein. However, as Figure 5.5f indicates most of the deer were eating predominately Western hemlock and Douglas fir, thus lowering the amount of protein they ingested; 10% took just less than 10%CP, 70% took 10-12%CP (mostly clustered around 10%CP) and 20% took 12-17%CP. At Squabb all of the deer took more than 10%CP but less than 12%CP (Figure 5.5f).

The deer at both sites fulfilled their requirements for this time of year; females 6-10%CP and males 6-11%CP (Table 5.3).

5.2.2.2 Calcium

In contrast to protein (Section 5.2.2.1) the data displayed in the triangular diagrams were summarised in Table 5.4 as there are only three levels of requirement, <0.3% calcium, 0.3-0.6% calcium (optimum) and >0.6% calcium representing the entire year (extrapolated by Adam, 1988). However the upper range is considered as being required for lactation and antler growth.

Table 5.4 Percentage of deer taking the different levels of calcium in each bimonth at each site, data taken from the triangular diagrams (Figures 5.6 - 5.9).

		Fallow deer sites		Roe deer sites	
	Requirement levels	**Brinken**	**Denny Lodge**	**Great Covert**	**Squabb**
JanFeb	< 0.3 0.3 - 0.6 > 0.6	- 100 0	5 70 25	- 100 0	- 10 90
MarApr	< 0.3 0.3 - 0.6 > 0.6	45 55 -	100 - -	50 50 0	- 0 100
MayJun	< 0.3 0.3 - 0.6 > 0.6	79 21 -	100 - -	33 67 -	95 5 -
JulAug	< 0.3 0.3 - 0.6 > 0.6	100 - -	100 0 -	72 28 -	- 79 21
SepOct	< 0.3 0.3 - 0.6 > 0.6	80 20 -	15 85 -	59 41 -	- - 100
NovDec	< 0.3 0.3 - 0.6 > 0.6	50 50 -	0 100 0	75 10 15	- 0 100

Fallow deer

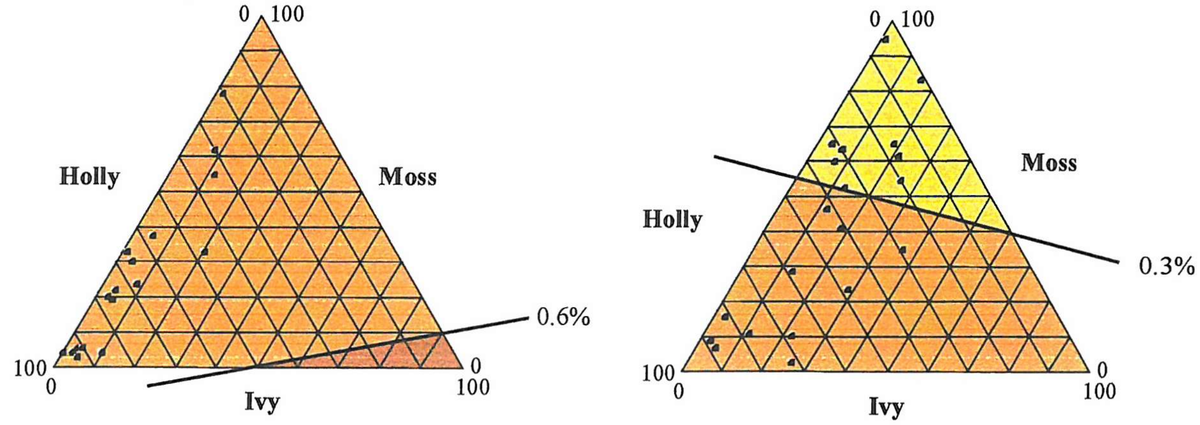
In JanFeb all the fallow deer (except 5% at Denny Lodge) took their requirement (Figures 5.6a and 5.7a). At Brinken just over half the deer (55%) continued this trend into MarApr (Figure 5.6b), whereas at Denny Lodge all the deer took less than 0.3% calcium, however there was no opportunity

Figure 5.6 Proportions of the three most commonly eaten plant species at Brinken each bimonth in relation to their calcium content (%) and with reference levels and deer (dots) indicated.

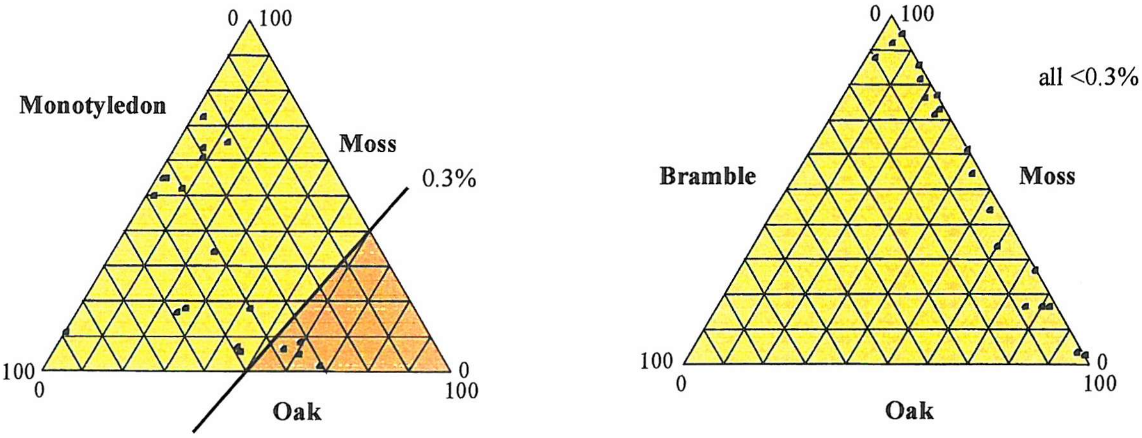
Legend:

🟨 <0.3% calcium 🟧 0.3-0.6% calcium 🟫 >0.6% calcium

a) JanFeb b) MarApr



c) MayJun d) JulAug



e) SepOct f) NovDec

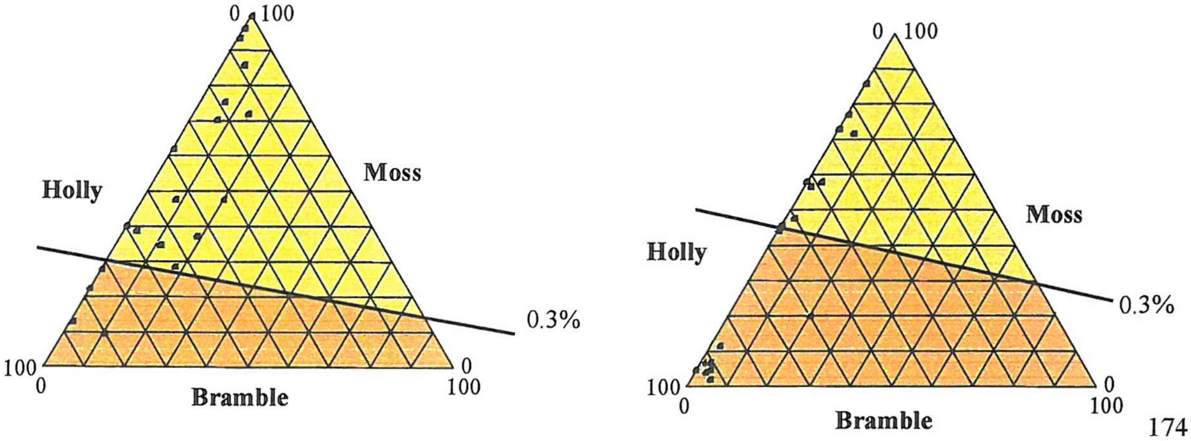
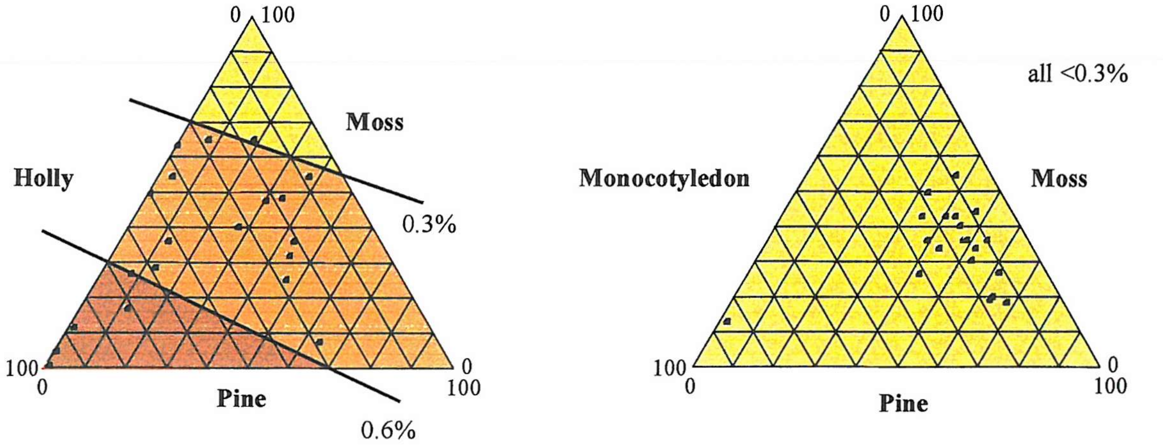


Figure 5.7 Proportions of the three most commonly eaten plant species at Denny Lodge each bimonth in relation to their calcium content (%) and with reference levels and deer (dots) indicated.

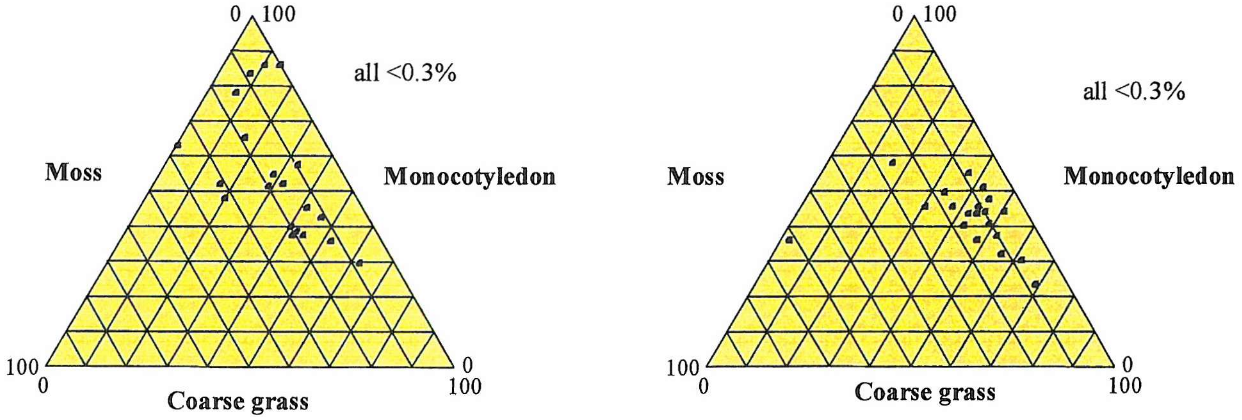
Legend:

🟨 <0.3% calcium 🟧 0.3-0.6% calcium 🟥 >0.6% calcium

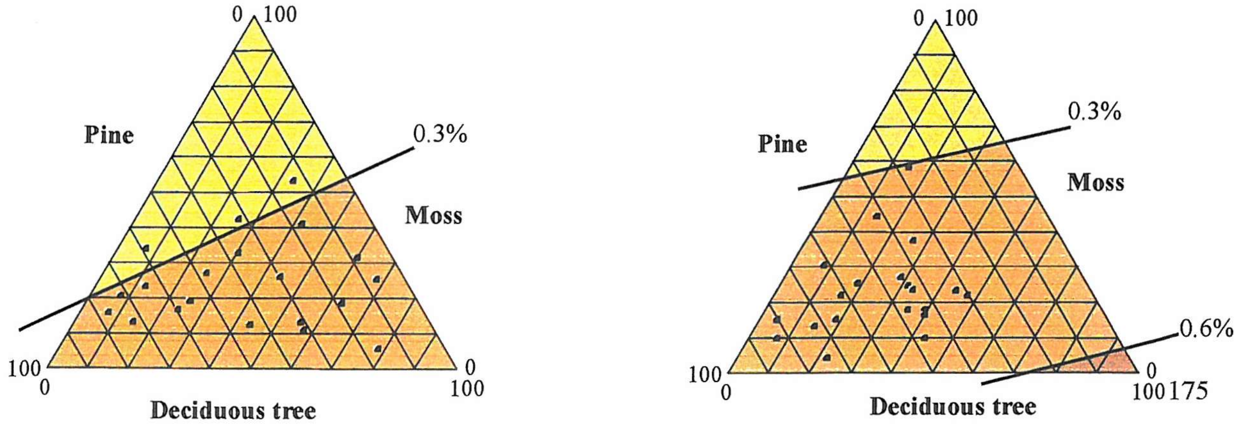
a) JanFeb b) MarApr



c) MayJun d) JulAug



e) SepOct f) NovDec



to take any higher (Figure 5.7b and Table A10.2, Appendix 10). By MayJun most of the deer at Brinken (79%) and all at Denny Lodge were taking less than 0.3% calcium (Figures 5.6c and 5.7c). This trend continued with all the deer at both sites taking less than their requirement. Brinken started to recover in SepOct with 20% of the deer fulfilling their requirement, increasing to 50% by NovDec (Figure 5.6f). Denny Lodge followed a similar pattern but more rapidly (SepOct: 85%; NovDec: 100%).

Roe deer

For the roe sites, there were five bimonths when the majority of the deer did not fulfil their calcium requirements (Figures 5.8 and 5.9). At Great Covert in MarApr, 50%; JulAug, 28%; SepOct, 41%; and NovDec, 25% of deer took 0.3-0.6% calcium or more, whilst the rest took <0.3% calcium. At Squabb all the deer in all bimonths, except MayJun, fulfilled their requirement. In MayJun only 5% took 0.3-0.6% calcium, the rest took <0.3% (Figure 5.9c). This decline would have been largely due to the low amounts of calcium available in the three most commonly eaten plant species (Table A10.2, Appendix 10).

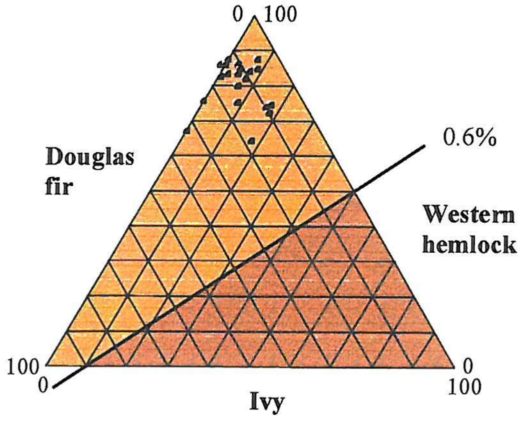
Roe generally took diets that fulfilled their requirement for calcium. This may simply have been because of the greater availability of plants with higher calcium content at the roe sites compared with the fallow sites. The soils at Brinken and Denny Lodge are acidic and the vegetation on such soils are known to contain less calcium than most soils (Kincaid, 1988), which may suggest why the vegetation at the roe sites contained more calcium. The majority of the fallow deer fulfilled their calcium requirements in the winter months (JanFeb and MarApr) which corresponds with the peaks in the calcium content of most plant species (Section 4.3.5.2).

Figure 5.8 Proportions of the three most commonly eaten plant species at Great Covert each bimonth in relation to their calcium content (%) and with reference levels and deer (dots) indicated.

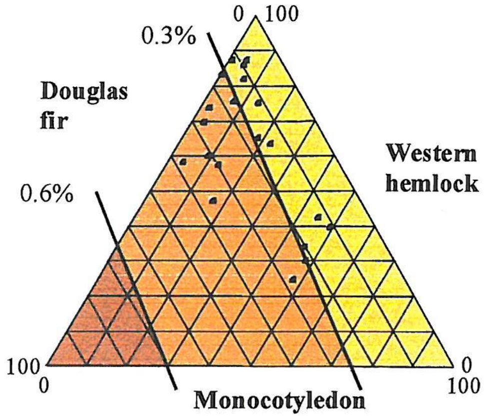
Legend:

🟨 <0.3% calcium 🟧 0.3-0.6% calcium 🟥 >0.6% calcium

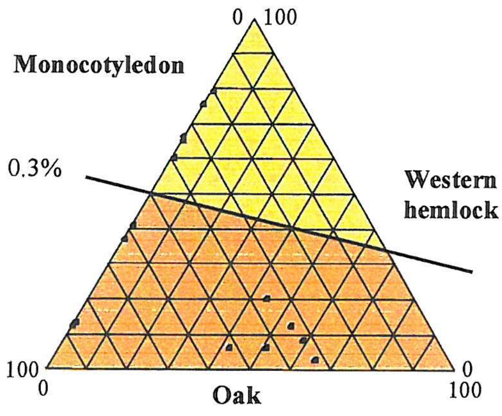
a) JanFeb



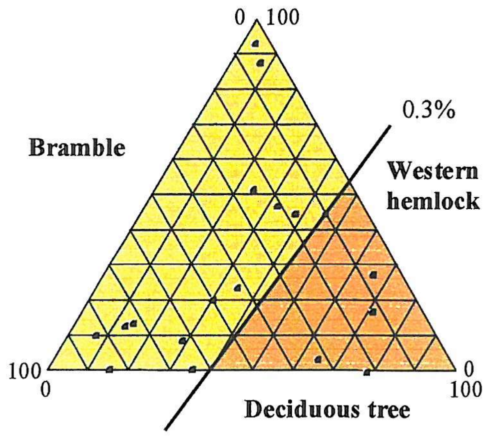
b) MarApr



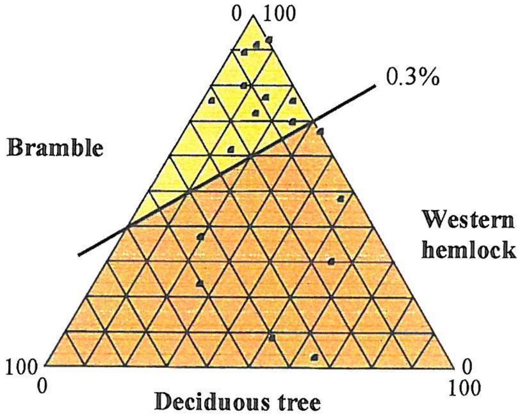
c) MayJun



d) JulAug



e) SepOct



f) NovDec

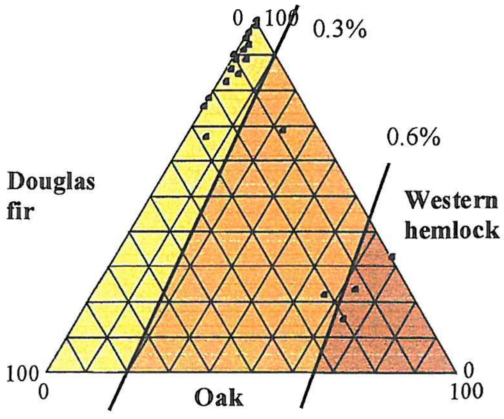
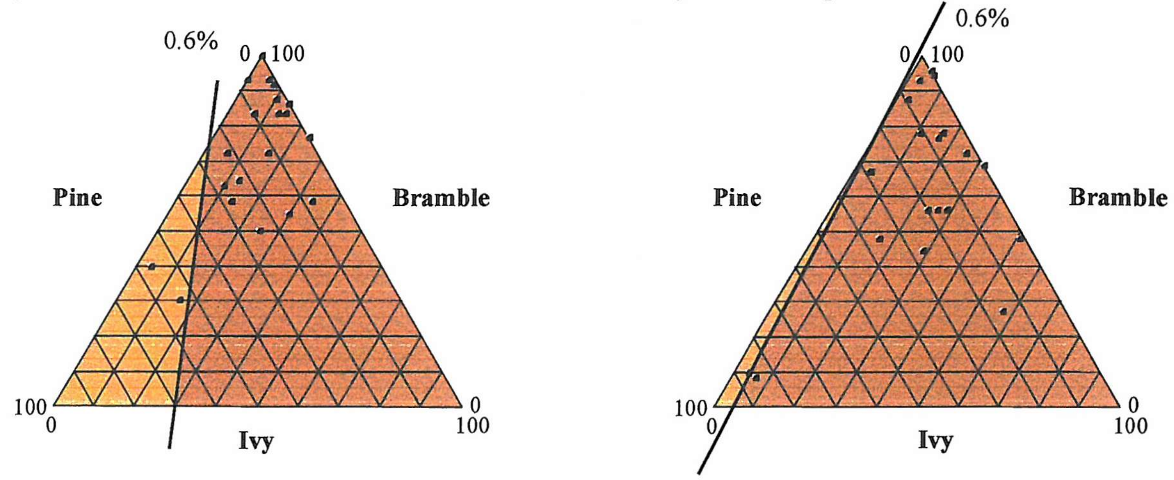


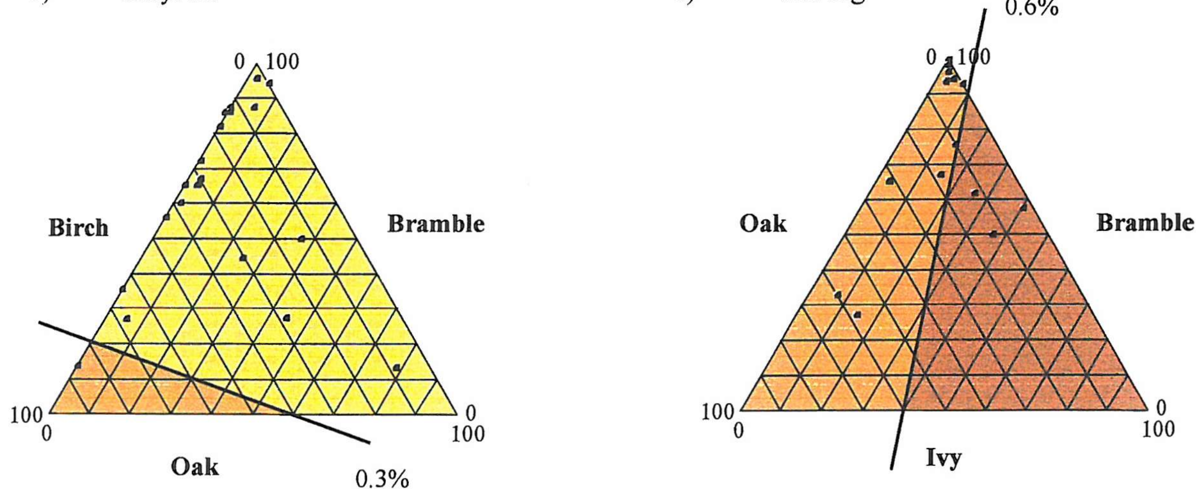
Figure 5.9 Proportions of the three most commonly eaten plant species at Squabb each bimonth in relation to their calcium content (%) and with reference levels and deer (dots) indicated.

Legend:

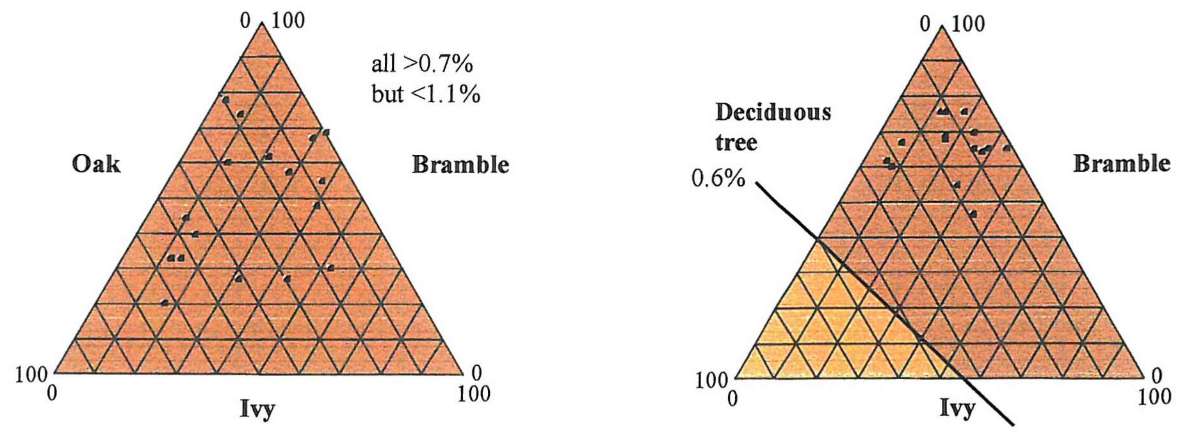
🟨 <0.3% calcium 🟧 0.3-0.6% calcium 🟫 >0.6% calcium


a) JanFeb

b) MarApr


c) MayJun

d) JulAug


e) SepOct

f) NovDec

5.2.2.3 Magnesium

As with calcium the results for magnesium have been summarised in Table 5.5 and data are compared to the requirement levels are <0.1% magnesium, 0.1-0.2% magnesium (optimum) and >0.2% magnesium (extrapolated by Adam, 1988). These requirements are assumed to be the same all year round.

Table 5.5 Percentage of deer taking the different levels of levels of magnesium in each bimonth at each site, data taken from the triangular diagrams (Figures 5.10 - 5.13).

		Fallow deer sites		Roe deer sites	
	Requirement levels	**Brinken**	**Denny Lodge**	**Great Covert**	**Squabb**
JanFeb	< 0.1 0.1 - 0.2 > 0.2	80 20 -	- 100 -	50 50 0	- 10 90
MarApr	< 0.1 0.1 - 0.2 > 0.2	0 100 -	75 25 -	70 30 -	0 32 68
MayJun	< 0.1 0.1 - 0.2 > 0.2	0 100 -	0 100 -	- 100 -	- 100 -
JulAug	< 0.1 0.1 - 0.2 > 0.2	95 5 0	0 100 -	11 33 56	- 50 50
SepOct	< 0.1 0.1 - 0.2 > 0.2	85 15 -	100 0 -	41 59 -	- 0 100
NovDec	< 0.1 0.1 - 0.2 > 0.2	56 44 -	0 100 0	0 95 5	0 0 100

Fallow deer

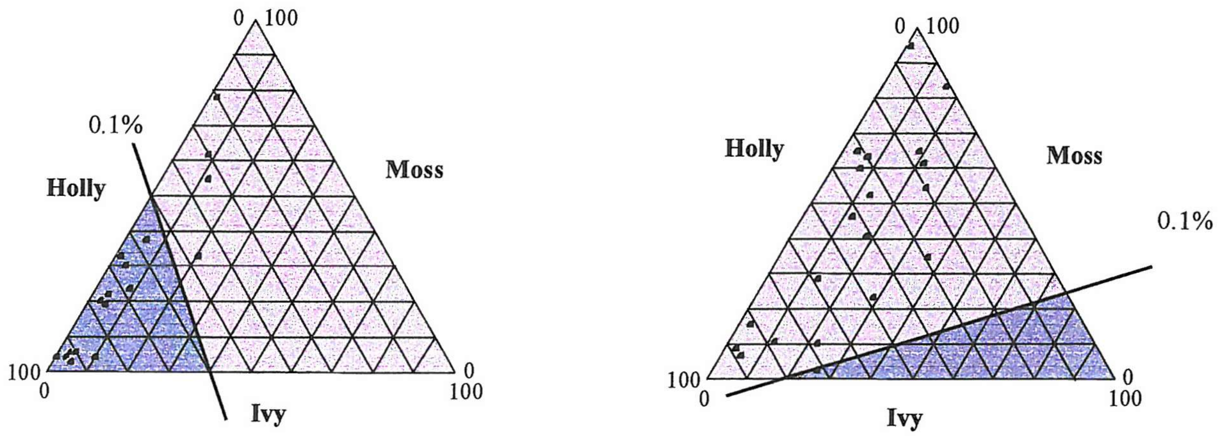
In JanFeb the majority of the deer at Brinken (80%) did not fulfil their magnesium requirement, despite the opportunity to do so (Figure 5.10a; Table A10.3, Appendix 10), whereas at Denny Lodge all of the deer fulfilled their requirement taking between 0.1 and 0.2% magnesium (Figure 5.11a). However, in MarApr at Denny Lodge most of the deer (70%) took less than 1% magnesium (Figure

Figure 5.10 Proportions of the three most commonly eaten plant species at Brinken each bimonth in relation to their magnesium content (%) and with reference levels and deer (dots) indicated.

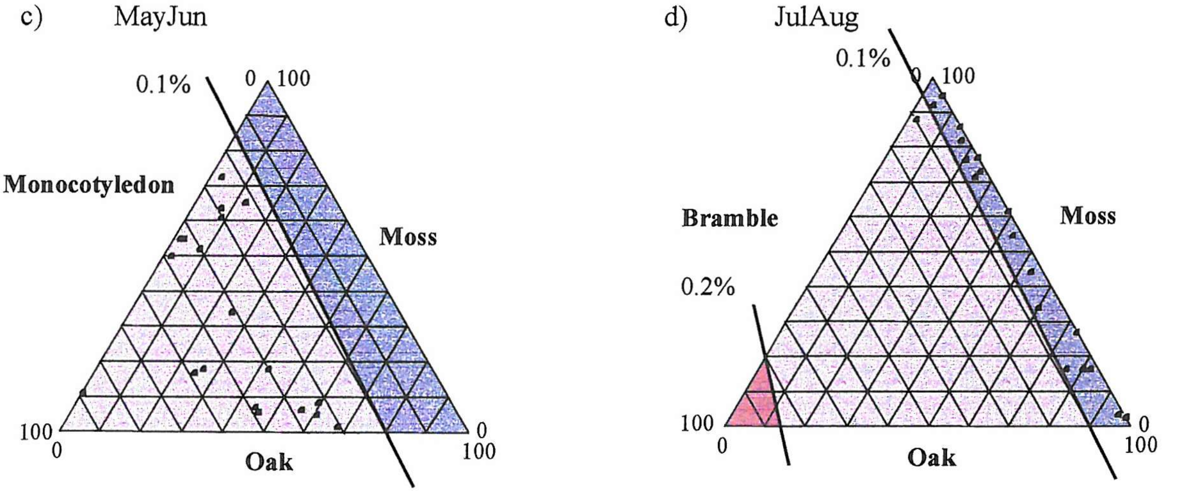
Legend:

▨ <0.1% magnesium ☐ 0.1-0.2% magnesium 🟥 >0.2% magnesium

a) JanFeb b) MarApr



c) MayJun d) JulAug



e) SepOct f) NovDec

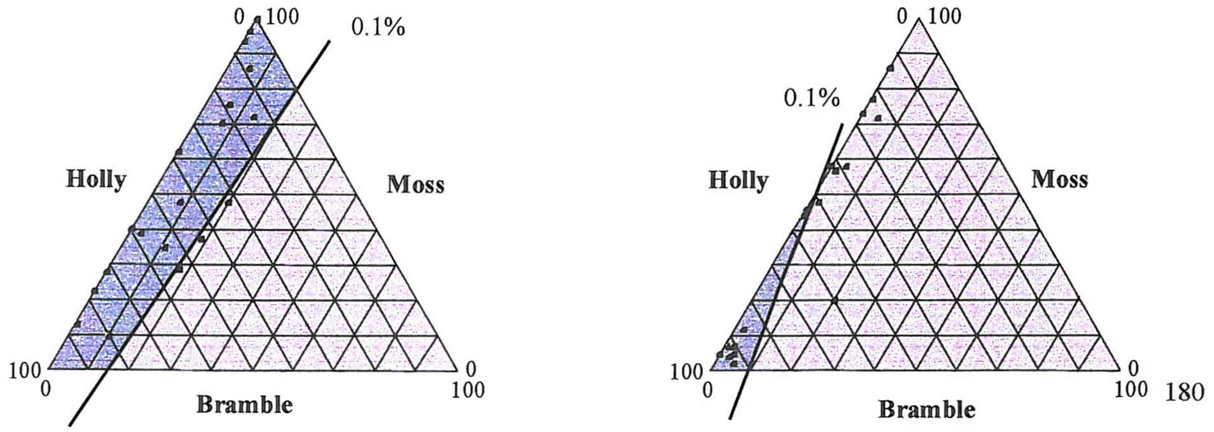
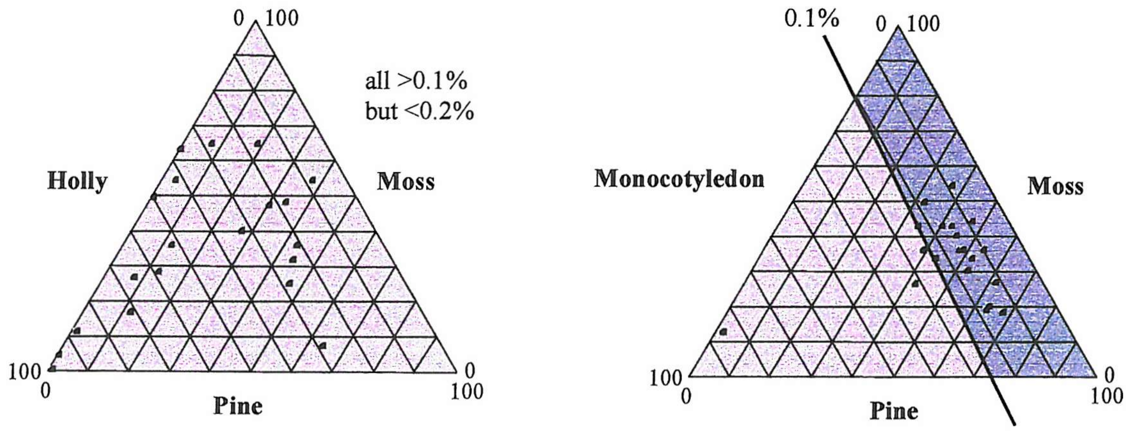


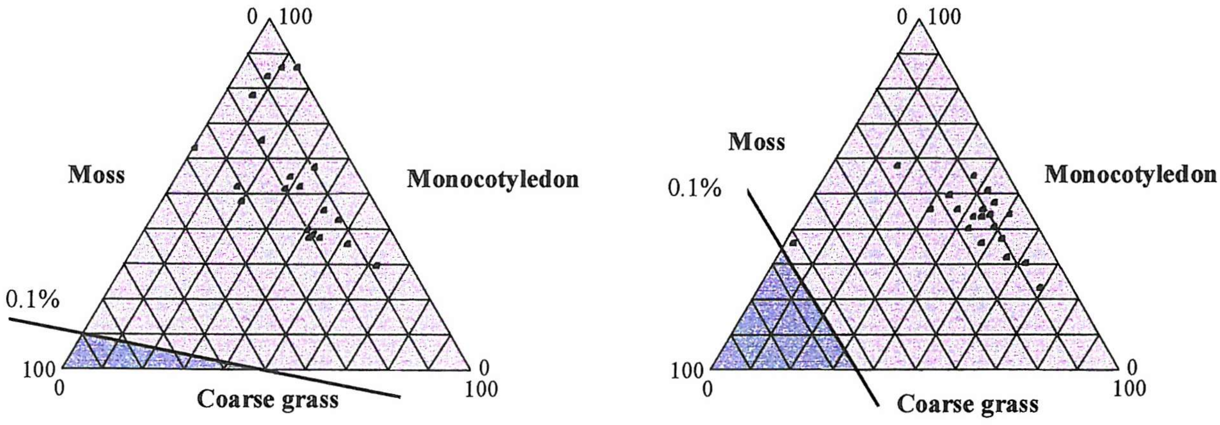
Figure 5.11 Proportions of the three most commonly eaten plant species at Denny Lodge each bimonth in relation to their magnesium content (%) and with reference levels and deer (dots) indicated.

Legend:

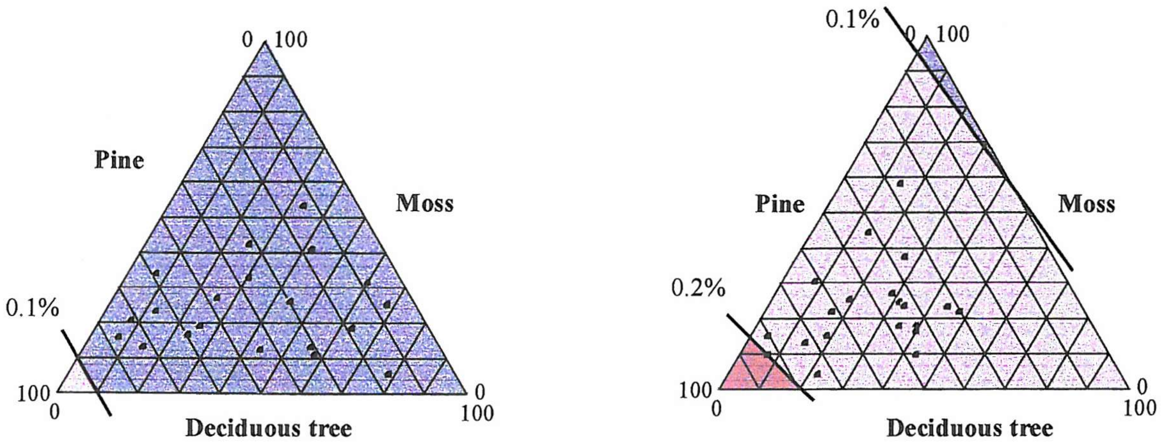
🟦 <0.1% magnesium ⬜ 0.1-0.2% magnesium 🟥 >0.2% magnesium


a) JanFeb

b) MarApr


c) MayJun

d) JulAug


e) SepOct

f) NovDec

5.11b) and all of the deer at Brinken took 0.1-0.2% (Figure 5.10b), fulfilling their requirement. For the remainder of the year at Denny Lodge the deer took 0.1-0.2% magnesium in MayJun, JulAug and NovDec, in SepOct all took less than 0.1% (Figure 5.11c-f). In contrast, at Brinken all fulfilled their requirement in MayJun (Figure 5.10c) but for the remaining bimonths most did not, despite the opportunity to take 0.1-0.2% magnesium (Table A10.3, Appendix 10).

Roe deer

At Squabb all the deer fulfilled their requirement for magnesium in every bimonth (Figure 5.13), most of the year a diet of more than 0.2% magnesium was available (Table A10.3, Appendix 10) and they appeared to take advantage of this. Again this could simply be due to the nutrient rich soil making it difficult to take a diet that did not fulfil their requirements. At Great Covert the majority (if not all) of deer took 0.1-0.2% magnesium or more in MayJun, JulAug, SepOct and NovDec (Figures 5.12c-f), for the rest of the year, although they had the opportunity to easily fulfil their requirements (Table A10.3, Appendix 10) the majority did not.

5.2.2.4 Potassium

Although no studies have looked specifically at the potassium requirement of deer there have been a number that have looked at other ruminants. Adam (1988) again used extrapolated data from domestic ruminants giving a requirement of 0.3% potassium. Other studies have suggested 0.5-0.6% for sheep and cattle (see Torgersen and Pfander, 1971) and 0.5-0.8% for sheep and cattle. The overall requirement range used in this study was 0.3-0.6% potassium, with levels <0.3%, 0.3%-0.6%.and >0.6%

All of the plant species at all of the sites contained more than the 0.3% potassium level and many took well in excess of 0.6% potassium (Table A10.4, Appendix 10). Therefore none of the deer could take a diet less than this figure thus none of them could be deficient in this particular nutrient.

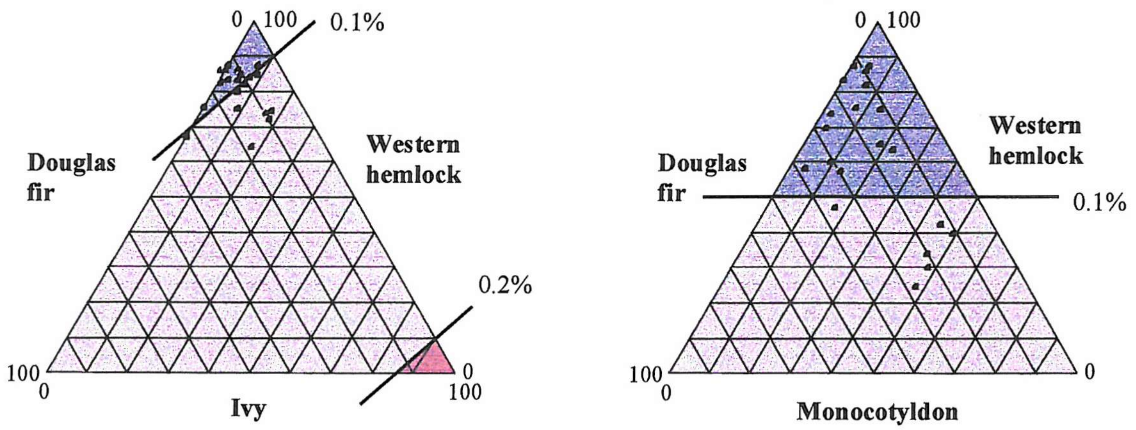
5.2.2.5 Phosphorus

The requirement for phosphorus is 0.2-0.4% phosphorus, as taken from the extrapolated requirements of domestic ruminants (Adam, 1988). This range is confirmed by Reinken *et al* (1990) (0.2 - 0.3% phosphorus) and French *et al* (1956) reported a minimum phosphorus requirement of 0.25% for

Figure 5.12 Proportions of the three most commonly eaten plant species at Great Covert each bimonth in relation to their magnesium content (%) and with reference levels and deer (dots) indicated.

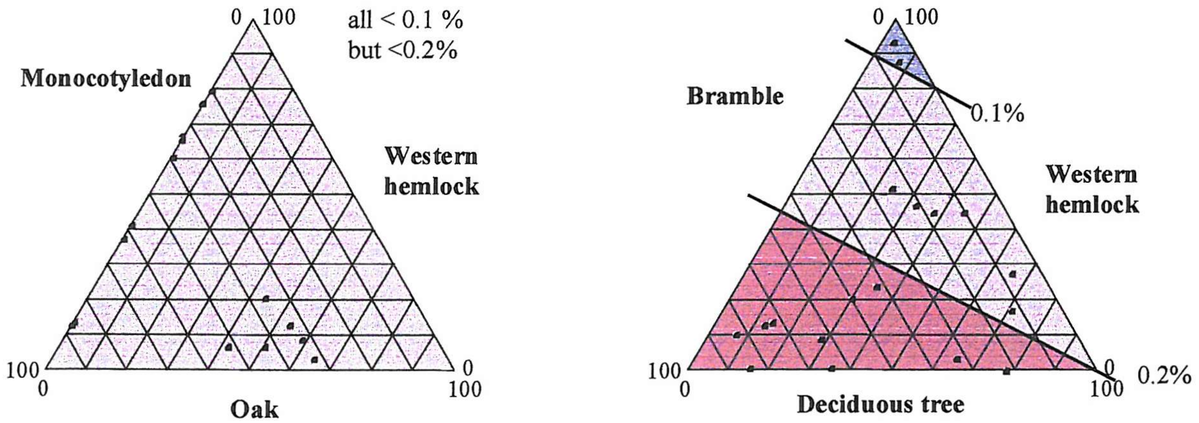
Legend:

▨ <0.1% magnesium ☐ 0.1-0.2% magnesium 🟥 >0.2% magnesium



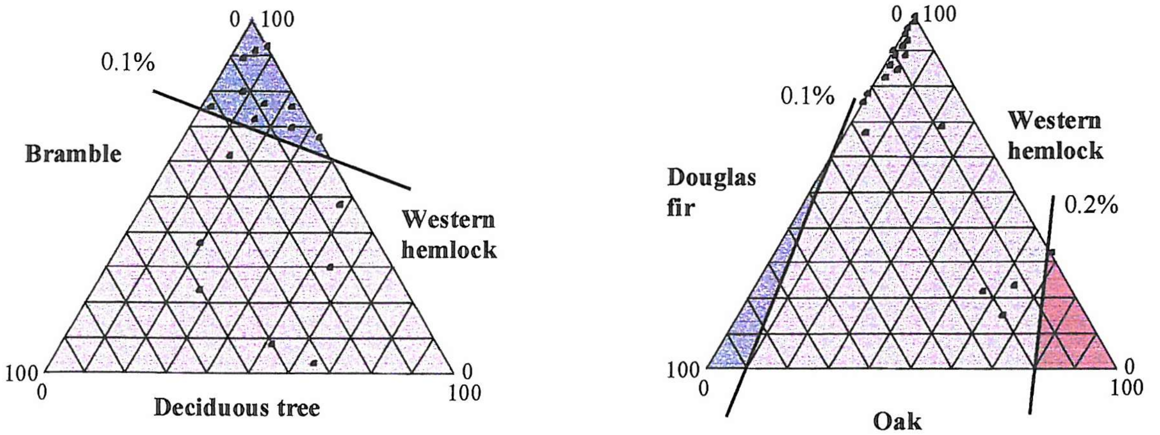
a) JanFeb

b) MarApr



c) MayJun

d) JulAug



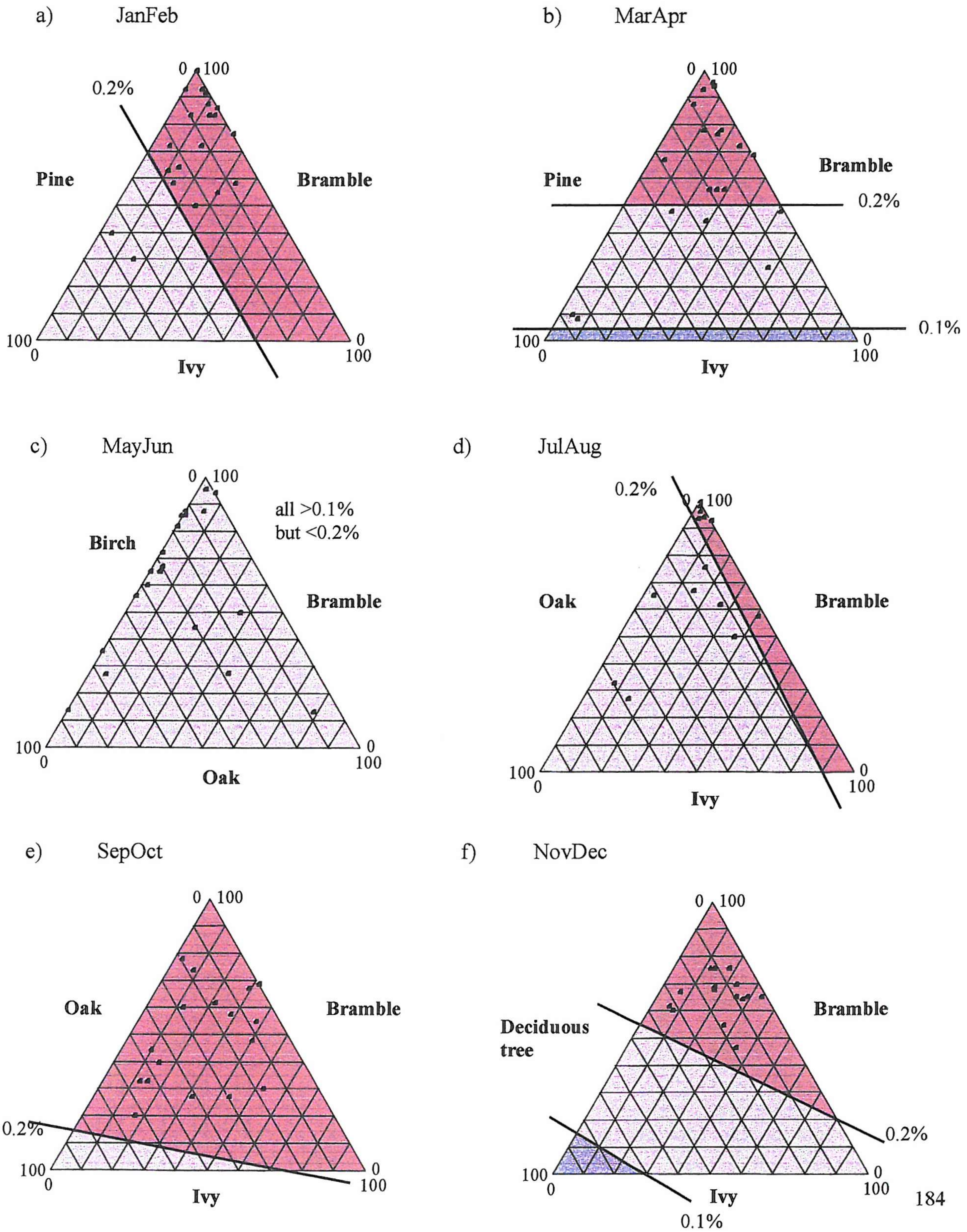
e) SepOct

f) NovDec

Figure 5.13 Proportions of the three most commonly eaten plant species at Squabb each bimonth in relation to their magnesium content (%) and with reference levels and deer (dots) indicated.

Legend:

🟦 <0.1% magnesium ◻ 0.1-0.2% magnesium 🟥 >0.2% magnesium



white-tailed deer. However, a study by Grasman and Hellgren (1993) found that for white-tailed deer phosphorus requirements changed seasonally; spring 0.16%; summer 0.11%; autumn 0.12%. The aforementioned range of 0.2-0.4% phosphorus was taken as being correct for the current study as data from Adam (1988) and Reinken *et al* (1990) have been used throughout. Only two of the plant species contained sufficient phosphorus: Monocotyledon at Denny Lodge in MarApr (0.26% phosphorus) and Bramble at Great Covert in JulAug (0.35% phosphorus), all of the other plants contained much less than 0.2% (Tables A10.5, Appendix 10). If the requirements produced by Grasman and Hellgren (1993) are correct, the majority (83%) of the recorded values were still very low (*i.e.* <0.11% phosphorus). There are several suggestions for this finding: apart from JulAug at Great Covert all the deer were likely to be deficient in this mineral; the requirement levels are incorrect; the method of analysis was defective.

5.2.2.6 Calcium:Phosphorus ratio

The low phosphorus content of the plants had serious repercussions on the calcium:phosphorus ratio. The suggested ratio for ruminants, including deer, is 1:1 to 2:1 (McDonald *et al*, 1981), if the phosphorus levels were to be believed for this study, most plant species had ratios that far exceeded the recommendations and went beyond levels considered safe.

5.3 CORRELATION BETWEEN PROPORTION OF PLANT SPECIES EATEN AND THEIR NUTRIENT CONTENT

5.3.1 Methodology

Proportions of plant species eaten were tested for correlations with their nutrient content over the year. This was in order to show whether or not the deer selected plants over the year based on their nutrient makeup. The criteria for inclusion into this analysis were species or groups that composed ≥2% of diet in any one bimonth and were detected in at least 5 bimonths (Appendix 4). The fallow and roe sites were analysed separately as the mean nutrient contents of grouped vegetation (*e.g.* Monocotyledons) were only valid if taken for each site individually (see Section 4.3.3).

5.3.1.1 Statistical methods used in data analysis

The statistical test used was Pearson's product moment correlation (see Fowler *et al*, 1998; Dytham, 1999). This correlation has strict assumptions about the two variables being analysed: both must be measured on a continuous scale and both must be normally distributed. The data sets under investigation here had both been measured on a continuous scale, as proportions. The distribution of each was not normal and so were normalised using the arcsine square root transformation, acceptable for data expressed as proportions (Dytham, 1999).

This correlation produces an r statistic in addition to a probability value, degrees of freedom are calculated as the number of pairs of observations minus two (Fowler *et al*, 1998), therefore in this study four degrees of freedom were usual representing the six pairs of bimonths minus two. Using this value critical levels of $r = 0.811$ at $P \leq 0.05$ and $r = 0.917$ at $P \leq 0.01$were taken (see Fowler *et al*, 1998). An r value (correlation coefficient) above these levels indicated a significant association (either positive or negative) between the two variables. Another useful statistic is the coefficient of determination and this is calculated as r^2 (Fowler *et al*, 1998). This indicates the percentage of the variation in one variable as explained by the other, the value may be given between zero and one or as a percentage, *e.g.* 0.83 or 83%.

Many of the correlations had probability values of $P>0.05$ as they were below the critical threshold of $r = 0.811$, the correlation coefficients were still large despite their lack of significance at this level and so were included and interpreted. Thresholds of $r \geq 0.7$ or $r^2 \geq 0.5$ were taken. The majority of these were significant at $P \leq 0.1$, although a few were just above this level. The level of $r^2 \geq 0.5$ was taken as a cut off point as below this most of the variation found in one variable would be attributable to something other than the second variable being measured, although it should be noted that a value of 0.5 indicates only a 50:50 chance of the second variable explaining the variation in the first.

5.3.2 Results

Tables 5.6a-b display the results from the Pearson's product moment correlation. Correlations where $r<0.7$ were not included for simplicity and all others were referred to as 'significant'.

Correlations are usually presented as scatterplots, however in this case bar and line graphs were used as these show the data from each bimonth. The plant species represented as graphs are those that

Table 5.6a Pearson's product moment correlations between nutrient content and proportion of plant in diet for **fallow deer** (in each cell value = *r*; ** = P<0.01, * = P<0.05, (*) = P<0.1, no star indicates P>0.1 but *r*>0.7, degrees of freedom = 4 or 3 for shaded cells; blank cells indicate *r*<0.7).

	Nitrogen		**Calcium**		**Magnesium**		**Potassium**		**Phosphorus**	
	Brinken	**Denny Lodge**	**Brinken**	**Denny Lodge**	**Brinken**	**Denny Lodge**	**Brinken**	**Denny Lodge**	**Brinken**	**Denny Lodge**
Holly			0.814 *	0.725	-0.808 (*)		-0.882 *			0.752 (*)
Moss	0.792 (*)		0.819 *		0.912 **	0.785 (*)	0.714			
***Carex* spp.**		-0.710		-0.752 *		-0.746 (*)		-0.773 (*)		-0.939 **
Ivy								-0.872 (*)		
Birch										-0.765
Herb		-0.787 (*)					0.845 *			
Coarse grass	-0.796 (*)							0.927 **	-0.749	
Monocotyledons	-0.807 (*)									
Sweet grass	-0.736 (*)						-0.777 (*)		-0.710	

Table 5.6b Pearson's product moment correlations between nutrient content and proportion of plant in diet for **roe deer** (in each cell value = *r*; ** = P<0.01, * = P<0.05, (*) = P<0.1, no star indicates P>0.1 but *r*>0.7, degrees of freedom = 4 or 3 for shaded cells; blank cells indicate *r*<0.7).

	Nitrogen		**Calcium**		**Magnesium**		**Potassium**		**Phosphorus**	
	Great Covert	**Squabb**	**Great Covert**	**Squabb**	**Great Covert**	**Squabb**	**Great Covert**	**Squabb**	**Great Covert**	**Squabb**
Ivy								-0.947 **	-0.932 **	-0.723
Bramble					0.917 *			-0.747 (*)	0.758 (*)	-0.864 *
Moss							-0.739 (*)			
***Carex* spp.**				0.937 **						
Calluna vulgaris			0.830 *				0.861 *			
Pine		-0.767 (*)		0.808 (*)						
Western hemlock								-0.784 (*)		
Birch					0.722					
Bracken										-0.801 (*)
***Juncus* spp.**					0.747 (*)		0.760 (*)		0.765 (*)	
Coarse grass			0.737 (*)							
Monocotyledons										0.756 (*)

correlated to more than one nutrient (Figure 5.14 - 5.19). Nutrients that were not correlated are included in the figures for comparison.

The results of the correlations will be described for each nutrient. A positive correlation (association) indicated that as the nutrient content increased so too did its consumption, thus from the graphs the two measures (proportion eaten and nutrient content) followed the same pattern over the year. Conversely, a negative correlation (association) indicated that the opposite was happening, as the nutrient content increased the consumption decreased and inverse patterns were noted on the graphs.

5.3.2.1 Protein (nitrogen)

Fallow deer

At Brinken a significant positive association was seen between the nitrogen content of Moss and the amounts in which it was eaten (Figure 5.14: $r_4 = 0.792$, $P<0.1$). The opposite was found for Coarse grass ($r_4 = -0.796$, $P<0.1$), Sweet Grass ($r_4 = -0.736$, $P<0.1$) and Monocotyledons in general ($r_4 = -0.807$, $P<0.1$).

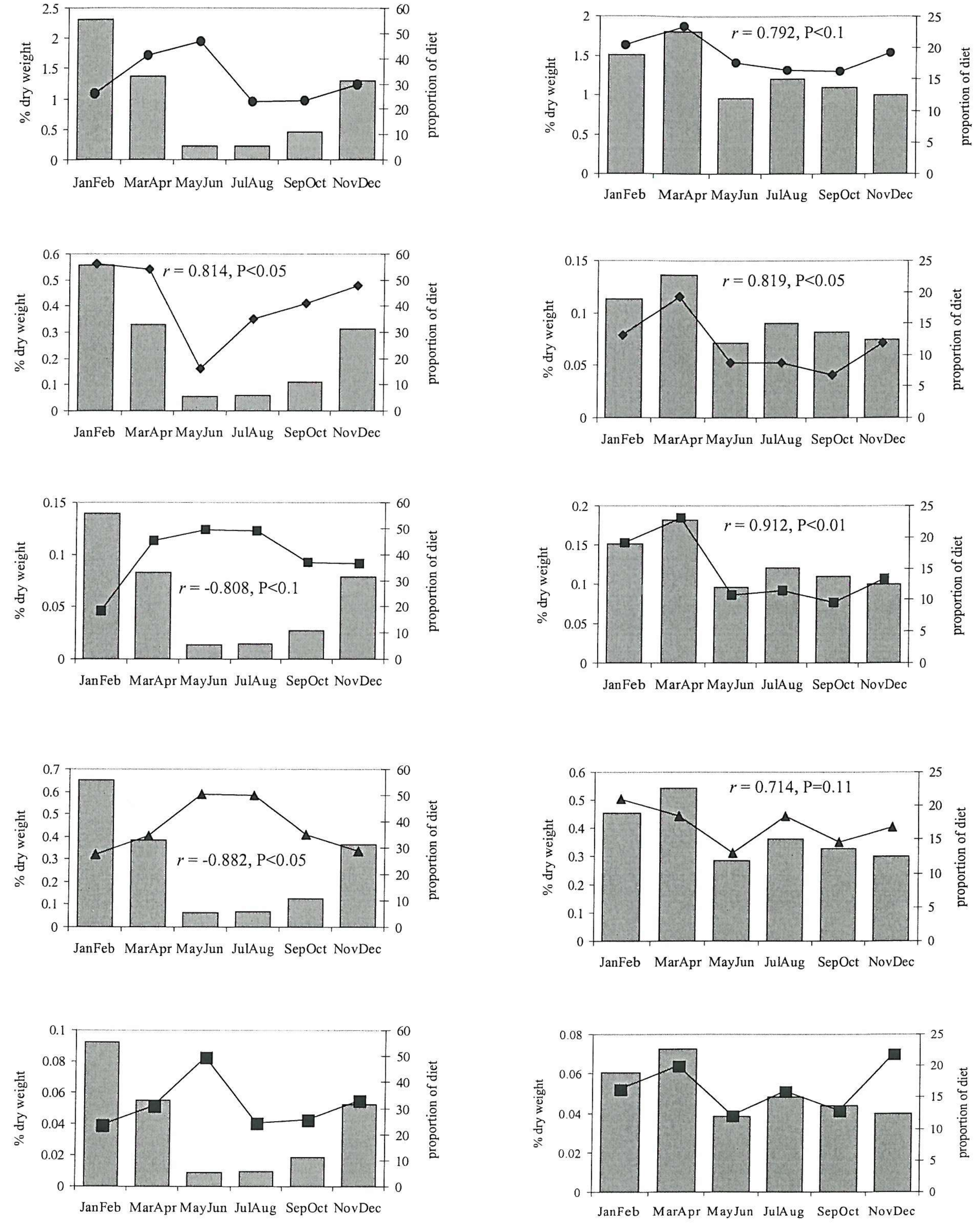
At Denny Lodge none of the relationships uncovered at Brinken were found. Only two associations were found here and both were negative: the group Herb ($r_4 = -0.787$, $P<0.1$) and *Carex* spp. (Figure 5.15: $r_3 =- 0.710$, $P = 0.114$).

Roe deer

The only relationship found at the roe sites, involving nitrogen content, was at Squabb and was a positive association for Pine ($r_4 = 0.767$, $P<0.1$).

Overall, where a correlation was found, the nitrogen content of plants was more usually negatively correlated with the amount eaten. However, all the r^2 values were relatively low (Table 5.6b) indicating that between 50 and 65% of the variation in the amounts of plants species eaten was explained by the nitrogen content. This means that although in most cases there was more than a 50:50 chance of this explanation, there may be another factor or factors involved. Although Moss was positively correlated at Brinken, there was no significant association at Denny Lodge.

Figure 5.14 Relationship between the nutrient content of Holly and Moss at Brinken and the proportions (bars) in which it was eaten over the year (● = nitrogen; ◆ = calcium; ▪ = magnesium; ▲ = potassium; ■ = phosphorus)


a) Holly

b) Moss

5.3.2.2 Calcium

Fallow deer

There were two positive associations between calcium content and amounts eaten at Brinken: Holly (Figure 5.14: $r_4 = 0.814$, $P<0.05$) and Moss (Figure 5.14: $r_4 = 0.819$, $P<0.05$). These correlations were stronger than those found for nitrogen. The r^2 values indicated 66 and 67% respectively of the variation in the amounts of Holly and Moss eaten were attributable to the calcium content.

The amount of Holly eaten also showed a positive association with the calcium content at Denny Lodge (Figure 5.15: $r_4 = 0.725$, $P = 0.103$), however the correlation coefficient was smaller giving an r^2 of 53%. In addition, Denny Lodge produced a relationship for *Carex* spp. (Figure 5.15: $r_4 = -0.752$, $P<0.1$).

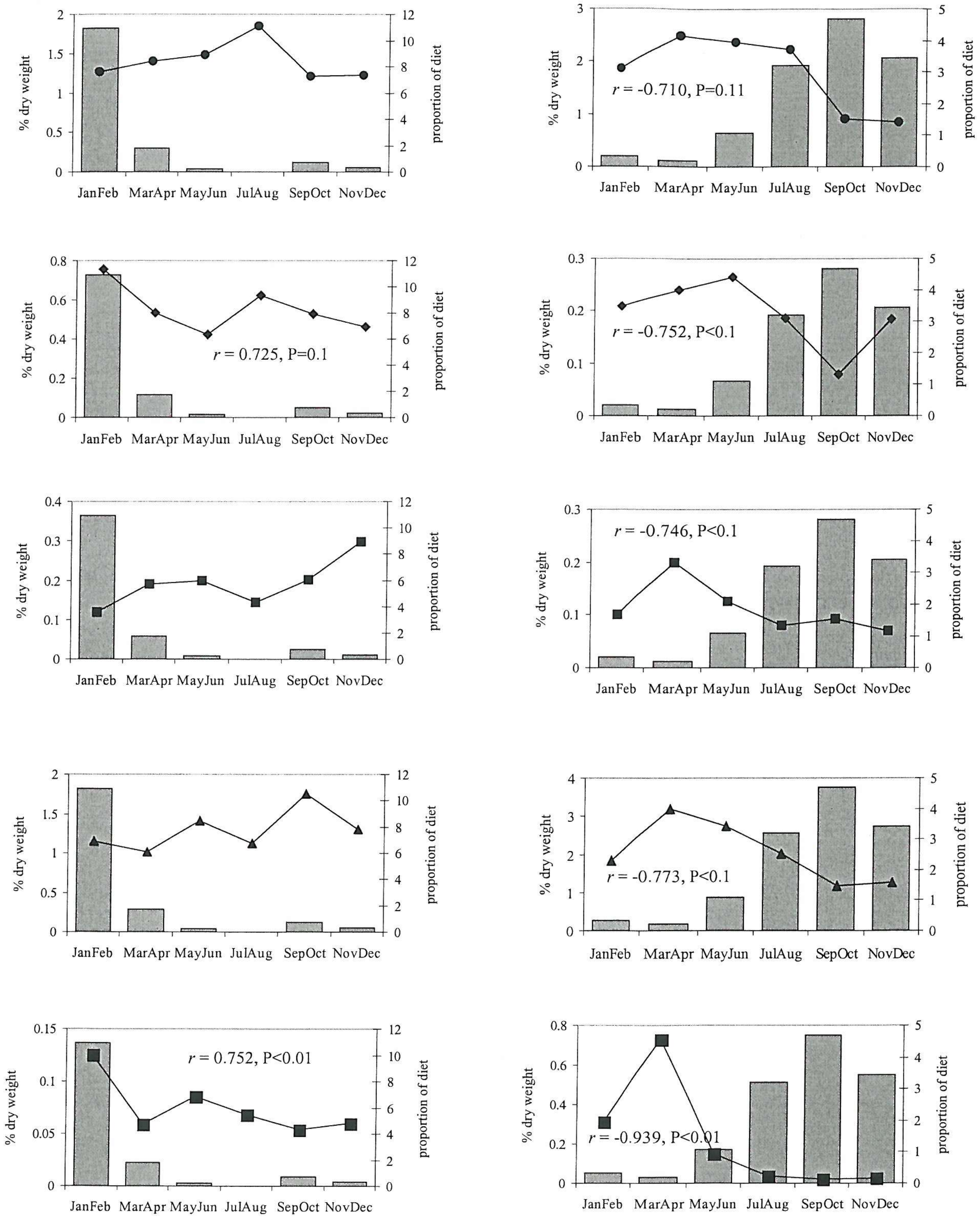
Roe deer

A significant positive association was found at Great Covert between the amount of *Calluna vulgaris* eaten and its calcium content ($r_4 = 0.830$, $P<0.05$) producing an r^2 value of 69%. Also at this site a further positive correlation was uncovered for Coarse grass ($r_4 = 0.737$, $P<0.1$), although the r^2 was only 54%.

In contrast to the negative association found at Denny Lodge (fallow site) between *Carex* spp. and calcium content, this relationship was highly significant and positive ($r_4 = 0.937$, $P<0.01$) at Squabb. The r^2 value indicated that 88% of the variation in the amount of *Carex* spp. eaten was explained by its calcium content. In addition to *Carex* spp. Squabb showed one other positive association, Pine, ($r_4 = 0.808$, $P<0.1$), yielding an r^2 of 65%, hence this percentage of the variation in the amount of Pine eaten was explained by the calcium content.

Overall, the majority of significant associations that involved calcium were positive, only *Carex* spp. at Denny Lodge was negative. Most of the species showing correlations occurred exclusively at one of the two fallow (Brinken or Denny Lodge) or roe sites (Great Covert or Squabb) and only Holly produced associations at both fallow sites.

Figure 5.15 Relationship between the nutrient content of Holly and *Carex* spp. at Denny Lodge and the proportions (bars) in which it was eaten over the year (● = nitrogen; ◆ = calcium; ■ = magnesium; ▲ = potassium; ■ = phosphorus)


a) Holly

b) *Carex* spp.

5.3.2.3 Magnesium

Fallow deer

Moss showed positive associations between the amounts eaten and the magnesium content at both fallow sites, (Brinken: Figure 5.14: $r_4 = 0.912$, $P<0.01$; Denny Lodge: $r_4 = 0.785$, $P<0.1$). Brinken displayed a higher level of significance with an r^2 of 83%, whereas for Denny Lodge this was reduced to 62%. The amount of Holly eaten at Brinken was found to be negatively associated with its magnesium content (Figure 5.14: $r_4 = -0.808$, $P<0.1$), 65% of the variation in the diet was explained by this nutrient content. At Denny Lodge *Carex* spp. was also negatively correlated to magnesium content (Figure 5.15: $r_3 = -0.752$, $P<0.1$).

Roe deer

None of the species that showed correlations between magnesium content and amounts eaten at the fallow sites were found at Great Covert or Squabb, in fact the latter site produced no correlations. Great Covert presented three notable associations, all of which were positive. Bramble showed a high positive correlation (Figure 5.16: $r_4 = 0.917$, $P<0.05$), the r^2 value indicates that 84% of the variation in the amount of Bramble eaten was accountable to magnesium content. *Juncus* spp. also gave a positive, although smaller, association ($r_4 = 0.747$, $P<0.1$) as did Birch ($r_3 = 0.722$, $P=0.168$).

Overall, most of the significant associations between amount eaten and magnesium content, at all sites were positive, only Holly at Brinken and *Carex* spp. at Denny Lodge were negative.

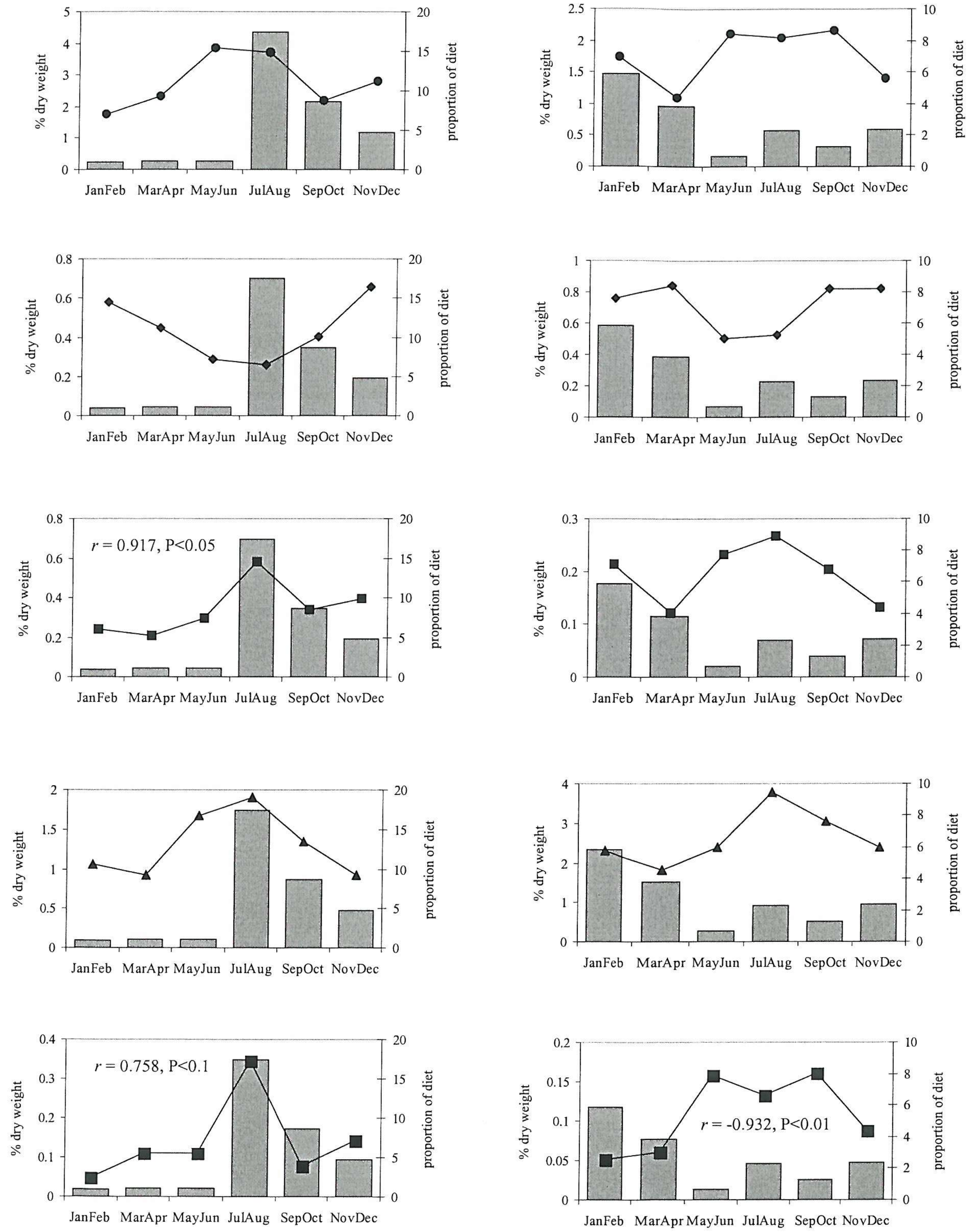
5.3.2.4 Potassium

Fallow deer

At Brinken there was a significant negative correlation between the amount of Holly eaten and its potassium content (Figure 5.14: $r_4 = -0.882$, $P<0.05$), this gave an r^2 value of 78%, the amount of variation in the amount of Holly eaten that was attributable to the potassium content. Another negative association at Brinken was for Coarse grass ($r_4 = -0.777$, $P<0.1$) Moss was positively associated (Figure 5.14: $r_4 = 0.714$, $P = 0.111$), as was the group Herb ($r_4 = 0.845$, $P<0.05$).

None of the relationships found at Brinken were seen at Denny Lodge. At this site, Coarse grass displayed a highly significant association between the amounts eaten and the potassium content ($r_4 = 0.927$, $P<0.01$), 96% of the variation in the amount eaten was attributable to the potassium content.

Figure 5.16 Relationship between the nutrient content of Bramble and Ivy at Great Covert and the proportions (bars) in which it was eaten over the year (● = nitrogen; ◆ = calcium; ■ = magnesium; ▲ = potassium; ■ = phosphorus)



a) Bramble

b) Ivy

the year could be explained by the potassium content. Ivy displayed a lower and negative correlation coefficient (Figure 5.15: r_4 = -0.872, P<0.1) and an r^2 of 60%. *Carex* spp. Was also negatively associated (r_3 = -0.773, P<0.1).

Roe deer

At Great Covert Moss produced a negative association (r_4 = -0.739, P<0.1) in the opposite direction but of similar size to the one at Brinken. Ivy at Denny Lodge displayed a negative correlation, which was also the case at Squabb, although the relationship there was highly significant (Figure 5.17:r_4 = -0.947, P<0.01); 90% of the variation in the amounts of Ivy present in the diet was attributable to the potassium content. *Calluna vulgaris* at Great Covert had a fairly high correlation coefficient (r_4 = 0.861, P<0.05) giving an r^2 of 74%, whereas *Juncus* spp. showed a weaker association (r_4 = 0.760, P<0.1). At Squabb Bramble and Western hemlock in the diet showed similar associations (Bramble: Figure 5.17: r_4 = -0.747, P<0.1; Western hemlock: r_4 = -0.784, P<0.1).

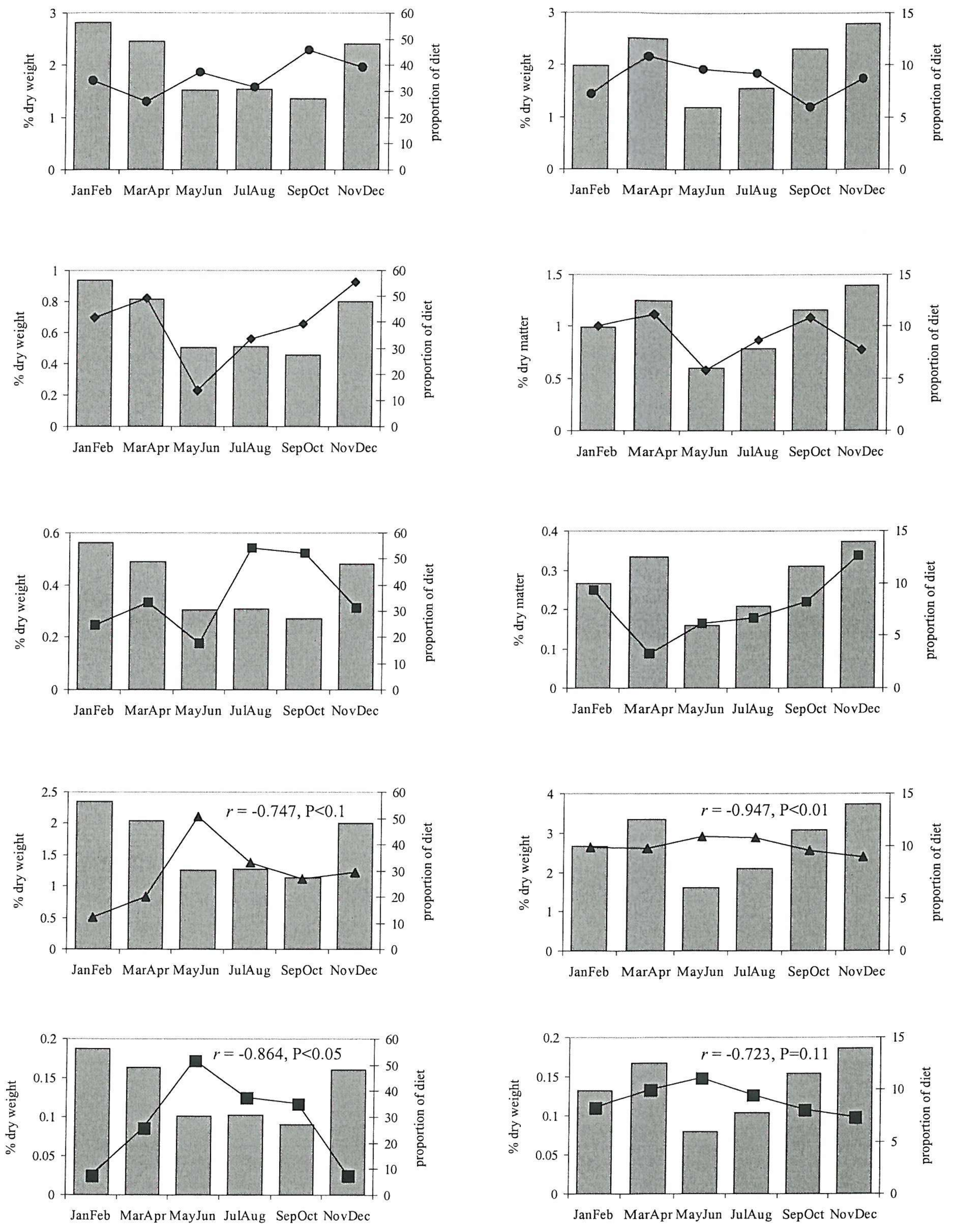
The potassium content was correlated to the amount consumed for several species, each site producing at least three significant correlations out of thirteen in total (phosphorus produced the same number see Section 5.3.2.5). The majority of these correlations were negative. Despite the high number of associations there were only two species that showed correlations at both fallow and roe sites; Ivy and Moss, and the relationship for Moss was reversed between the two species of deer. Also, there were no species that showed significant correlations at both fallow sites or both roe sites that would allow for comparison within deer species.

5.3.2.5 Phosphorus

Fallow deer

At Brinken, consumption of Coarse grass and Sweet grass were negatively correlated to their phosphorus content (Coarse grass: r_4 = -0.749, P = 0.145; Sweet grass: r_4 = -0.710, P = 0.114), however their r^2 values were relatively small, 56 and 50% respectively, suggesting that the variation in the amounts of grasses eaten may quite easily be explained by something other than phosphorus content. At Denny Lodge for Holly (Figure 5.15: r = 0.752, d.f. = 4, P<0.01) and Birch consumption (r_3 = -0.765, P = 0.131), showed correlations to phosphorus content. The most significant association was at Denny Lodge for *Carex* spp. (Figure 5.15: r_4 = -0.939, P<0.01), this relationship was negative and the r^2 value indicated that 88% of the variation in the amount of *Carex* spp. eaten by the deer at Denny Lodge could be explained by its phosphorus content.

Figure 5.17 Relationship between the nutrient content of Bramble and Ivy at Squabb and the proportions (bars) in which it was eaten over the year (● = nitrogen; ◆ = calcium; ■ = magnesium; ▲ = potassium; ■ = phosphorus)



a) Bramble

b) Ivy

Roe deer

Two plant species at Great Covert that showed associations between the amounts eaten and their phosphorus content were also seen at Squabb, these were Ivy and Bramble. At Great Covert Ivy displayed a highly significant negative correlation (Figure 5.16: r_4 = -0.932, P<0.01) and at Squabb the magnitude of the association was reduced (Figure 5.17: r_4 = -0.723, P=0.105). Bramble at Great Covert produced a positive relationship (Figure 5.16: r_4 = 0.758, P<0.1) whereas at Squabb the association was negative (Figure 5.17: r_4 = -0.864, P<0.05). At Squabb a higher percentage of the variation in the amount eaten was attributable to the phosphorus content, albeit in the opposite direction to Bramble at Great Covert. The other associations produced at each site were for *Juncus* spp.at Great Covert (r_4 = 0.765, P<0.1) and Bracken (r_4 = -0.801, P<0.1) and Monocotyledon (r_4 = -0.756, P<0.1) at Squabb.

Overall, there were the same total number of associations produced involving the phosphorus content as there were for the potassium content. Five of these were for the same species or groups, although the direction of the association was not always the same. Two species at the roe sites allowed intra-deer species (or inter-site) comparison as they occurred at both sites, one produced similar results for both sites (Ivy) and the other (Bramble) displayed a reverse relationship at each site. This inverse relationship at the two sites was of concern as there could be several possible reasons: the deer were behaving the complete opposite at each sites, Bramble was very different at the two sites or the method of analysis is not adequately reliable for looking at diet selection (see Section 5.4.4).

5.4 DISCUSSION

5.4.1 Consideration of plant digestibility

The plant chemical analysis (Chapter 4) gave total nutrient concentrations of the vegetation. These would not have been completely available to the deer, as each plant species is digested to a different degree. It is very difficult to combine published digestibility values (Section 4.3.5.6) with the current data, no conversion factors are available therefore assumptions are made regarding the effect of digestibility on the nutrient intake of the sampled deer.

In the first analysis (triangular diagrams) the majority of vegetation combinations at each site and for each bimonth contained at least one species that was considered to be moderately (30-50%) if not highly digestible (>50%). Invariably these were the species eaten in the highest amounts. In this case the values discussed were considered accurate although they may have been slightly overestimated. Certain bimonths were of greater concern. At Denny Lodge, a lot of Moss and Pine (conifer) were eaten particularly in the summer, both of these are very low in digestibility (20.3% and 20.5% repectively). Therefore only the summer months (MayJun, JulAug) at Denny Lodge were considered free of the implications of digestibility as Pine was not eaten. At Great Covert, similarly the deer ate a lot of conifers (Western hemlock and Douglas fir), and again only in the summer, when they were not eaten, was digestibility not a problem. Brinken and Squabb were not thought to be affected by digestibility to any meaningful degree, most of the plant species were readily digestible, except for Moss at Brinken but this was not eaten in the same amounts as found at Denny Lodge.

Combining the digestibility information with the correlation data is more problematic as the whole year was considered for each species of vegetation. Some plants vary in their digestibility over the year so generalising their digestibility was not possible. However, certain species are more consistent, Holly, Ivy and Bramble are all highly digestible, whereas Moss and conifers are both poorly digested. Unfortunately no such conclusions could be drawn for the other plants included in the correlation analysis, however their digestibilities over the year will be explored if necessary.

Reference will be made to the digestibility values throughout the following discussions of the triangular diagrams (Section 5.4.2) and correlations (Section 5.4.3).

5.4.2 Consumption of nutrients in this study in relation to published requirements

The nutritional requirements used in this part of the study were taken from Adam (1988) and Reinken *et al* (1990) as they were the most comprehensive, moreover the latter study gave protein requirements specifically for fallow deer. However, reference to other studies will be made in the discussion of each nutrient. These include studies involving other species of deer; chemical analysis of what they were observed eating or their known preferred forages. This discussion centres around the three main plant species eaten in each bimonth and explores nutrient requirements in terms of only the deer eating these. The advantages and disadvantages to this method are discussed in Section 5.4.4. This analysis dealt with the question "are fallow and roe deer getting sufficient nutrients to meet their known requirements?". This question will be addressed separately for each nutrient.

5.4.2.1 Protein (nitrogen)

There were no occasions when the deer could have taken a diet that was less than 6%CP. Although Reinken *et al* (1990) state that a diet of 6%CP is adequate in the winter many researchers do not agree. Work in North America on white-tailed and black-tailed deer have suggested that a diet of less than 7%CP and certainly less than 6%CP severely impairs the function of the rumen (Dietz, 1965). Diets of between 4.6-6.6%CP have seriously limited antler growth in white-tailed deer (French *et al*, 1956) and black-tailed deer have been reported to not live long on a diet of less than 5%CP (Einarsen, 1946). In these accounts it is not stated how long the deer need to be eating such a diet before they will be harmed.

There appears to be a fine line between deficiency and maintenance. Researchers state 7-8%CP as being adequate for body maintenance (French *et al*, 1955; Magruder *et al*, 1957), whereas others give this range as being suboptimal for physical development (Murphy and Coates, 1966). When all findings are considered the optimum range for weight gain is wide, 11-18%CP (French *et al*, 1956; Magruder *et al*, 1957; McEwan *et al*, 1957; Holter *et al*, 1979). As there is some argument between what is acceptable and what is not, the protein requirements supplied in Table 5.3 will be used in comparison with the findings of this study.

The protein requirements recorded by Adam (1988) and Reinken *et al* (1990) imply that in most cases in this study there was sufficient protein available from the three most commonly eaten plants for both males and females to fulfil their needs in each bimonth at each site. There were six occasions when none of the plants yielded adequate protein. Five of these were, unsurprisingly, for females as they have higher requirements due to the demands of gestation and lactation. They occurred in MarApr and JulAug at Brinken and Squabb, also JulAug at Denny Lodge, digestibilities in these bimonths would not have any substantial effect on the values obtained. The other occasion when there was insufficient protein was in MarApr at Brinken but for males as well as females (maximum = 11.7%CP). On these five occasions the deer were deficient in protein according to their physiological requirements. However, there were other times of the year when the deer were taking diets lacking in protein despite the availability of much higher concentrations.

At Brinken, the female fallow deer would have been deficient in protein throughout late pregnancy, birth and lactation, only in MayJun were half of them consuming enough protein to sustain them adequately, the digestibilities would not have had any effect in this bimonth. In SepOct there was ample protein available from the three most common plant species, however both the males and females took much lower amounts, the reason for which was unclear, however the digestibility could have had a marginal effect as Moss (poorly digested) was included in the diet. In JanFeb and NovDec both sexes took sufficient protein which was unaffected by the digestibility values.

At Denny Lodge the males had adequate protein all year round, however the females were deficient in MarApr and JulAug. Digestibility in MarApr was also low therefore the females were likely to be more deficient than first thought, however in JulAug there was no effect. Over the remainder of the year sufficient protein was consumed from the three most commonly eaten plant species.

Both males and females at Great Covert had protein deficient diets in MarApr despite adequate levels being apparently available, however digestibility would have been a problem here, seriously reducing the concentrations available. To have taken an acceptable diet the deer would have had to eat predominantly Monocotyledons. The fact that roe are concentrate selectors, and not grazers, was probably one of the reasons they took less. Also Monocotyledons were comparatively low in availability. Most of the females in MayJun and JulAug also took a low protein diet compared to their requirement. The main reason for this was probably the high availability yet very low protein content and digestibility of Western hemlock. Many of the deer were taking this predominantly, despite the appearance of highly digestible Oak leaves in MayJun (26.8%CP) and Bramble in JulAug (23.2%CP). Some deer did take more Oak or Bramble and had mean protein intakes of 21% (MayJun) and 22%CP (JulAug). This value is around that required by fawns for maximum growth (Ullrey *et al*, 1967; Smith *et al*, 1975) however these deer were not likely to be fawns as they would just have been born around this time of year and also small faeces were not collected (Section 2.2.2). More fittingly, these animals could have been lactating females. For the rest of the year deer generally took diets that fulfilled their requirements, whether male or female.

As mentioned above, MarApr and JulAug at Squabb followed the same scenario as Brinken; there was not enough protein in the three most commonly eaten foods to satisfy the requirements of females and digestibility was not a contributing factor. The majority of males were also deficient during these bimonths, but could have taken an adequate diet if they had eaten more Ivy in MarApr and more Oak in JulAug. During the latter bimonth the males were rutting and are known to eat very little at this time (Chapman and Chapman, 1975), as a result they may not actively select a nutritious diet, which could explain the apparent deficiency. Sufficient protein was supplied over the rest of the year to fulfil the needs of both males and females.

The main time of year that both fallow and roe deer were deficient in protein was MarApr to JulAug, predominantly females but also males at the roe sites. This finding for females was of concern as this is a crucial time in their life cycle when a deficiency could have pronounced consequences for the mother and her offspring. The increases in protein requirements during lactation are very dramatic because milk solids contain about 27% protein (Schingoethe *et al,* 1988) and protein deficiencies could lead to poor milk quality and poor growth of the offspring. The interpretation of the summer period was less reliable due to the recalculation of the proportions of main plant species in the diet (see Section 5.4.4). However, of the species not included in the top three none were eaten in significant amounts that had high enough protein concentrations to have alleviated the deficiencies witnessed at this time of year. The highest overall concentration of nitrogen was found in Birch (Figure 4.5, page 135) and this species was not eaten in substantial amounts. It may also have been included into the group Deciduous tree, but this group was not eaten in conspicuous amounts (other than where it featured in the top three) to suggest any changes to the overall protein intake over this critical time. It is a possible that the deer were eating forage outside of the study area that were richer in nutrients, but this cannot be corroborated.

Other studies looking at the protein content of preferred forage species found that the requirements could not be met in the winter (Short *et al,* 1966; Torgerson and Pfander, 1971) however, over the winter time (NovDec and JanFeb) in this study all the deer, males and females, at each site took diets that fulfilled their requirements. Admittedly the needs were substantially less in the winter but the protein content of the plants also decreased at this time (Figure 4.6, page 135). The problems encountered with the recalculation of the proportions were not an issue for the winter data as most of the diets at this time were made up (in the vast majority) of the three most commonly eaten species. There has been a suggestion that protein is a limiting factor for deer in winter and that selection occurs at this time (Parfitt, in Putman, 1986a). This study can corroborate this suggestion, in that both fallow and roe deer appeared to be selecting specific foods that satisfy their requirements in the winter.

5.4.2.2 Calcium

Unfortunately calcium could not be analysed in the same detail as protein because the requirements for deer are not really known and have only been extrapolated from those of domestic ruminants (Adam, 1988). They are assumed to be more or less the same at all times of the year despite physiological state. However, the requirement for calcium does increase during late pregnancy and lactation for females, during antler growth for males and for young growing animals. Studies with red deer have shown that the total amounts of nutrients, including calcium, secreted by females in milk (0.22% calcium) is slightly less than the amounts deposited in the antlers (Adam, 1988). Reduced antler size is a reflection of poor general nutrition not just poor nutrient intake (Adam, 1988). If this intake falls below the requirement deer, like sheep, can draw upon nutrients from their bones, which they then replenish at other times of the year (Adam, 1988).

The calcium requirement was taken as 0.3-0.6%. The overall pattern of calcium content over the year was the inverse of nitrogen (protein), there was a decrease over the summer period, increasing as the year progressed (Figure 4.9, page 137). This increase is due to senescence of the leaves (Ekins, 1989). Therefore if there was going to be a period of calcium deficiency or low intake it was expected to be during summer.

This pattern was witnessed at Brinken and Denny Lodge. At Brinken, although there was sufficient calcium available in the three most commonly eaten species in MayJun, JulAug and SepOct, the vast majority of deer took less than their requirement (*i.e.* <0.3% calcium), digestibility over this time would not have considerably reduced the available calcium. At Denny Lodge, the plant species in MarApr, MayJun and JulAug all produced diets deficient in calcium, none contained more than 0.19% calcium. Digestibility in MarApr could have added to the problem of deficiency but not in the other two bimonths. Over the rest of the year, at both sites, there was adequate calcium to fulfil the requirements and most, if not all, of the deer took advantage of this. The behaviour of the deer at Brinken over the summer suggests that they do not have the same need for calcium as at other times of the year. Over the period of antler growth and lactation in fallow deer (May-September: Table 5.3), the majority of the deer at Brinken and Denny Lodge fell short of the 0.3-0.6% calcium requirement and digestibility was not an issue. Assuming that fallow deer females provide their offspring with milk of similar calcium content to that of red deer (0.22% calcium) and the males' antlers require a similar amount, the deer at these two sites may have become calcium deficient during this time. The summer months were the time of lactation and antler regrowth, so it was assumed that calcium would be needed in high amounts, however the results suggested that these needs were fulfilled from calcium stores within the body. This would be most detrimental to the females as gestation and lactation are considerably more demanding in mineral costs than antler growth (Brown, 1990 cited in Grasman and Hellgren, 1993). Pletscher (1987) estimates that female mineral requirement is approximately 57% higher than male because calcium (and phosphorus) incorporated into foetal tissue and milk is 4-6 fold that required for an average set of antlers.

At Great Covert there was apparently more than adequate calcium available all year round, however, the majority of deer took less than their requirement in JulAug, SepOct and NovDec. However, the digestibilities in the latter two bimonths suggests that the amounts of calcium available would have been lower than first thought. In the other winter bimonth, JanFeb, it appears that the requirement was fulfilled with all the deer taking 0.3-0.6% but the low digestibilities of the conifers indicate that the high concentrations were probably not so readily available. In MarApr, when digestibility was also low, half took less than 0.3% and the remainder obtained required levels. In MayJun, when concentration began to wane but digestibility increased, most of the deer were still taking the required amounts. For Squabb, there were comparatively very high concentrations of calcium available from the three most commonly eaten plant species in each bimonth, except for MayJun. Thus, all of the deer fulfilled or exceeded their requirement in every bimonth apart from MayJun. Male roe deer regrow their antlers at a different time to fallow, in JanFeb (Staines and Ratcliffe, 1991), therefore at both sites as the deer were taking their suggested requirement or above, this may well have been contributing to the generation of new antlers at the time instead of using stores as suggested for fallow. Female roe lactate over the same time as fallow (Chapman and Putman, 1991; Staines and Ratcliffe, 1991) but often for longer, at Squabb the requirements were covered adequately from JulAug onwards but the females at Great Covert would have been deficient although calcium was available and in spite of the assumed increase in demand.

As stated earlier, the roe deer took much higher quantities of calcium throughout the year, including the period of antler growth and lactation (Table 5.3). Assuming the same demands are put upon roe deer as red deer, the animals at Great Covert and Squabb should not have become calcium deficient. This is despite the calcium requirement not being fulfilled at Squabb in MayJun, the period of early lactation. This current study considers that enough calcium was taken the rest of the year that these deer should not have experienced any problems.

With the exception of Denny Lodge, generally there was sufficient calcium available to the deer all year round, this was also found by Dietz (1962) and Torgerson and Pfander (1971) who chemically analysed preferred forage species. Despite the availability the deer did not always choose a diet that fulfilled their requirements, unfortunately the researchers mentioned did not look at what actual deer were eating.

5.4.2.3 Magnesium

As for calcium, the requirements for magnesium were extrapolated from those of domestic ruminants (Adam, 1988), they were not reported for different times of the year or the different sexes. The suggested levels for magnesium were 0.1-0.2%. The overall magnesium content increased as the year went on, peaking in late summer (Figure 4.12, page 139). Generally, the content of all three most commonly eaten species in each bimonth at each site yielded sufficient magnesium that any deficiency could be avoided, this has also been reported by Kincaid (1988). This was the case at Squabb, the deer took their recommended requirement or more in every bimonth. At Great Covert the majority of the deer also did this from MayJun to NovDec, however in the latter bimonth digestibility was low thus the deer may have found it harder to take their requirement. In JanFeb and MarApr half or more of the deer took a diet of less than 0.1%, and were thus deficient, digestibility was also low at this time. For the fallow deer at Denny Lodge, MarApr was also a deficient bimonth as was SepOct, however this latter bimonth contained the least amount of calcium overall. It was impossible to take more than 0.1% unless the deer had only eaten Pine, but as this is very low in digestibility it is likely that they could not have achieved their requirement. At Brinken, the pattern was very different; although there was sufficient magnesium all year round, the requirement was only fulfilled in MarApr and MayJun (when digestibility was not a problem), for the rest of the year most of the deer took less than 0.1%. They may not have been greatly deficient most of the year as sheep have been recorded as taking between 0.04 and 0.08% magnesium (Kincaid, 1988), but during the period of lactation magnesium requirements increase two or four fold and therefore they were likely to be taking an inadequate diet for this purpose.

There was very little similarity between the sites for magnesium intake in terms of its availability and requirement, therefore no conclusions can be drawn other than if the requirements were correct, they were easily fulfilled by the vegetation eaten.

Overall the deer at Brinken may have become deficient in magnesium particularly over the winter months with the build up from the summer and the demands from lactation and antler growth (Table 5.3). For deer at the other sites there was no reason to suggest they may be deficient in magnesium.

5.4.2.4 Potassium

Deficiency in potassium was not a possibility at any of the four sites in any bimonth, all took a diet that contained 0.3% potassium or more. The digestibilities of the plant species involved should not have decreased the concentrations greatly to create deficiencies of this nutrient. The highest levels were available in MayJun and JulAug. Torgerson and Pfander (1971) found that forage eaten by white-tailed deer contained adequate potassium in the summer and winter months. This was also found by Short *et al* (1966) working with mule deer. Ekins (1989) chemically analysed plants eaten by cattle, ponies and deer in the New Forest and found that the recommended potassium levels were always attainable.

There are two reasons why the potassium content of the plants was always high: firstly potassium is resistant to leaching and is the dominant cation in the soil (van Soest, 1994) and secondly, in plants it is assumed to be almost completely available to ruminants (Kincaid, 1988), as 99% is in the cell soluble fraction (Kincaid and Cronrath, 1983). Despite there being no reported deficiencies in the current or past studies, they can occur in specific circumstances. Of more concern at the study sites was excess intake as some of the plants eaten contained extremely high amounts (*e.g.* Monocotyledon 3.73%; Ivy 2.64%). Kincaid (1988) states that large intakes of potassium are associated with the disease grass tetany (caused predominantly by deficiencies in magnesium coupled with high levels of potassium and protein). This was certainly a consideration here particularly for the fallow deer (predominantly grazers) with the high potassium content of the Monocotyledon group. Also, digesting large amounts of potassium can reduce magnesium absorption (Greene *et al*, 1983) which could have had a serious impact on the deer at Brinken in the winter when they where already deficient in magnesium, however the opportunity to take in high amounts of potassium was reduced in the winter at this site.

5.4.2.5 Phosphorus

There is evidence to suggest that the phosphorus analysis of the vegetation may have been incorrect, although no obvious reason can be given for this. The values obtained were low compared to published levels (Table A9.5, Appendix 9) and fall below the published requirements (Adam, 1988; Reinken *et al*, 1990). If the measured values were correct all the deer at all the sites (except JulAug for Great Covert) would have been deficient in this nutrient. Although phosphorus is recognised as being the most commonly deficient nutrient after sodium (Kincaid, 1988), deficiency on the scale suggested in this study was extremely unlikely.

The calcium:phosphorus ratios confirmed that the measured levels may be unreliable. The recommended ratio is 1:1 to 2:1 (McDonald *et al*, 1981), however ruminants are known to be able to tolerate much higher levels, up to 6:1 (Kincaid, 1988). Above this level the phosphate absorption is reduced which is critical when phosphorus is deficient (Kincaid, 1988), as suggested in this study (Section 5.3.2.5). Researchers investigating the chemical content of deer forage found ratios close to 2:1 (Dietz *et al*, 1962; Dietz, 1965; Radwan and Crouch, 1974). One study found much wider ratios ranging to 20:1 in summer and winter (Torgerson and Pfander, 1971). Despite this, the number of exceptionally high ratios found in this study did not suggest the phosphorus had been correctly analysed.

As a result further discussions of phosphorus in the diet selection by deer must be carefully considered.

5.4.3 Correlation between proportion of plant species eaten and their nutrient content

By correlating amounts eaten with nutrient content of specific forage this analysis dealt with the question "are fallow and roe deer selecting plants according to their nutrient content?". A strong correlation, either positive or negative, could indicate selection for or against a specific plant species. This is explored by examining the nutrients collectively for each forage item. The likelihood of selection occurring is then discussed in view of other factors, particularly availability.

The majority of correlations for nitrogen, potassium and phosphorus were negative, indicating that when the concentrations of these nutrients increased over the summer their consumption (in whichever plants) decreased and the reverse occurred in the winter. For calcium and magnesium most correlations were positive, suggesting that as the concentrations increased so too did the intake of those plants with high calcium and magnesium. In most basic terms this could be described as selection however this analysis was compounded by the fact that most correlations only occurred for plant species at one fallow site or one roe site allowing no corroboration of the findings. This could be due to chance, however it was assumed not to be the case because of the numbers of these single correlations. There could be a number of possible explanations. The deer may be exhibiting different foraging strategies, there could be differences in availability or differences in chemical content between sites. It could indicate a lack of sensitivity with this method of looking at diet selection or it could suggest that the chemical analyses were unreliable.

The latter reason has already been demonstrated as unlikely in Chapter 4. With the exception of phosphorus, the values obtained compared very favourably with published data. For the present study it is assumed that the Pearson's product moment correlation analysis was the correct tool to look at diet selection. This suggested that the chemical content of most species was very different at the two fallow and roe sites or the deer were using different foraging strategies probably employing a number of factors including plant availability and chemical content.

Having only one correlation for a plant species at one fallow or roe site does not give a clear indication of what may be happening. This may be more easily addressed by looking at those occasions where correlations were produced at both fallow or both roe sites. There were only four instances when this occurred: calcium content of Holly and magnesium content of Moss for fallow deer; phosphorus content of Bramble and Ivy for roe deer. Holly, Moss (fallow sites) and Ivy (roe site) showed similar associations at both sites but for Bramble the direction of the correlation was reversed. Each relationship will be described in an attempt to unravel whether or not the correlations can be attributed to diet selection, starting with the species that showed the same direction of correlation.

At Brinken and Denny Lodge (fallow sites) positive correlations were produced between the calcium content of Holly and its consumption. Holly is highly digestible throughout the year and therefore the results of the correlation would not be affected as all the calcium within should have been made available to the deer. At Brinken, Holly was much more available than at Denny Lodge, which probably contributed to its high presence in the diet, up to 56% at Brinken and only 11% at Denny Lodge. Selection of Holly at Brinken based on its availability was suggested for the winter (Section 3.3.4). The correlation with calcium content also suggests selection for Holly in the winter months. The calcium content is lowest in MayJun which coincides with the time when the lowest amounts of Holly were eaten, consumption increases as the calcium content increases, hence the positive correlation. Calcium has already been shown in Chapter 4 to increase into the winter months in agreement with other researchers (Ekins, 1989). At Denny Lodge the lowest calcium content for Holly also occurred in MayJun remaining low into JulAug. Consumption decreased at this time and then increased very slightly in comparison to Brinken, this may have been due to the lower availability at Denny Lodge thus resulting in the weaker correlation coefficient. However the patterns were very similar and suggest that **selection for Holly was occurring at both sites in the**

winter months but that availability played a key role.

Brinken and Denny Lodge also produced positive correlations between consumption and the magnesium content of Moss. In this case the availability at each site was very similar, however when compared to consumption, selection was suspected in the summer at Brinken (Section 3.3.4). Moss was eaten in greater amounts at Denny Lodge than at Brinken (maxima = 42% and 23% respectively). At Brinken peak consumption was in MarApr when magnesium concentration was at its highest, the pattern of intake and magnesium content were very similar hence the highly significant correlation. The pattern at Denny Lodge was slightly different, the peaks in consumption and magnesium content occurred earlier, in JanFeb, decreasing to the lowest levels in the summer months. JanFeb at Brinken, although not the peak bimonth, also showed high consumption and magnesium content therefore it can be suggested that **Moss was being selected for in the winter bimonths by fallow deer at both sites**. This was the opposite of what was suggested by the availability measures alone. Although the evidence has been presented to suggest selection, Moss is very poorly digested and therefore the amounts of magnesium used in the correlation would have been much lower, however as this would have been the case at both sites there may still be an argument for selection occurring.

As mentioned earlier there was a strong possibility that phosphorus had been incorrectly analysed due to the low concentrations found. As there was no clear evidence for this and the seasonality of this nutrient has been shown to consistent with the findings of others (Section 4.4.2.2: Ekins, 1989), it was therefore included in the correlation analysis which considered the pattern of concentration over the year not the actual content.

The phosphorus content and consumption of Ivy produced negative correlations at Great Covert and Squabb. Ivy is highly digestible all year round and the availability at Squabb was much greater than at Great Covert, however this alone did not suggest selection was occurring in either summer or winter for this species (section 3.3.4). However, most likely as a result of the increased availability, the roe deer at Squabb ate more Ivy over the year than those at Great Covert. The phosphorus concentration of Ivy was highest over MayJun, JulAug and SepOct at Great Covert where there was more variation than at Squabb which peaked in phosphorus content in MarApr and MayJun. When phosphorus was at its highest the consumption of Ivy was at its lowest, hence the negative correlations; at Great Covert this was stronger probably because of the increased variation. As the same patterns were found at both sites and the maximum phosphorus concentrations were very similar, it can be suggested that **there was selection against Ivy in the summer bimonths.** Tixier *et* *al* (1997) also found that roe deer avoid ivy in the summer and prefer it in the autumn/winter.

At Great Covert and Squabb the correlations produced between Bramble and phosphorus were in opposite directions, this was the only case of this. Bramble is generally highly digestible all year round and this coupled with its availability at Squabb were probably the main reasons all the roe deer at Squabb ate it predominantly for most of the year (Appendix 4). In contrast at Great Covert it was eaten mostly in JulAug and occurred in the diet of all the deer around JulAug and SepOct. The peak in phosphorus at Great Covert occurred in JulAug which coincided with an increase in the digestibility of Bramble and a peak in its consumption, hence the positive correlation. Therefore, it may be suggested that the **deer at Great Covert were selecting for Bramble in the summer** based on the increased phosphorus content through increased digestibility. At Squabb selection was not evident on this basis, they ate it all year round and in MayJun JulAug when the phosphorus levels were highest the roe deer ate less Bramble than at other times of the year and increased their Oak consumption, hence the negative correlation. Therefore it is more likely that the deer were selecting for Oak at this time (Oak was not analysed due to insufficient degrees of freedom) and not actively selecting against Bramble. However, although the availability at Squabb was only slightly higher than at Great Covert, selection in the summer and the winter was suggested when availability was compared to the intake (Section 3.3.4).

These inter-site comparisons with either similar or very different correlations have indicated how complex selection can be. Although it was suggested on a number of occasions, it is clearly not due to nutrient content alone and is multifaceted. However it was clear that both fallow and roe deer were selecting diets at different times of the year (not simply eating species in proportion to their abundance), particularly in the winter, and that the nutrient content played a fundamental role. Unfortunately there were insufficient inter-site comparisons to determine if certain nutrients have more of a function in diet selection than others. Also there is insufficient published data for comparison. Parfitt (in Putman 1986a) suggested that selection for protein may occur in winter, although this was not confirmed here, winter was certainly the period when selection was most likely.

As most of the correlations were only at one site and comparisons were not possible, it was difficult to say whether or not these showed selection by the deer. However, it does appear that using Pearson's product moment correlation clearly measured some degree of selection but not necessarily based on one nutrient alone.

5.4.4 Relative value of the two analysis methods used

The two methods of analysis were used primarily as they were addressing different research questions. There were advantages and disadvantages for both methods.

Correlation analysis uses a statistical test therefore the results should be more robust than the conclusions that can be drawn from the first method. However, because of the low number of degrees of freedom the critical levels were high and so the probability levels were increased to $P \leq 0.1$. The correlation used all plant species or groups that were eaten in abundance (*i.e.* ≥2% of the diet in any one bimonth), whereas the first method looked only at the three most common for each bimonth. Because the deer ate more than three species, for each bimonth the proportions had to be recalculated, this was particularly unreliable in the summer as the species used to calculate the diagrams often occurred in relatively small amounts and recalculation as proportion of 100% could be misleading. The winter triangular diagrams were more reliable.

The correlations allowed trends over the year to be statistically analysed. The triangular graphs gave information for each bimonth. These graphs also provided information on what the individual deer were selecting whereas the correlations looked at what all the deer were doing at a particular site. The only disadvantage with the triangular graphs was that any information on species that were not in the top three most abundant in the diet was lost, even though their nutrient content may have been high. However, these graphs showed that using the three most abundant species alone generally provided enough of the nutrients at each physiological stage (Section 5.4.2).

The manner in which the triangular diagrams were presented allowed the nutritional status of the deer (*e.g.* whether or not they were deficient) at each site for each bimonth to be described and compared to the literature. This was not possible with the correlation data.

The overall intake of each nutrient could have been measured for each bimonth but it is believed that this would not have provided a clearer understanding of what the deer were doing. Plant species occurring as <2% of the diet would contribute very little to the overall nutrient content for a particular bimonth, therefore a different arbitrary cutoff value would simply be imposed. By creating the triangular diagrams it has been possible to plot each individual deer and the requirements for each nutrient thereby providing a more comprehensive picture of diet selection than that provided by one total figure for nutrient content each bimonth.

5.4.5 Summary and general discussion

Digestibility is considered to be an integral part in determining nutritive value (Barnes, 1965 cited in Drodz, 1979), although it has been very difficult to incorporate into the current study, reference to it has been made where possible. Digestibility only became relevant when the deer were already taking a deficient diet, in such cases low digestibility indicated that they were taking an increased deficient diet than first thought. The other occasions digestibility became important was when the deer were thought to be taking a diet containing a lower nutrient concentration than was available to them, incorporating digestibility showed that the higher levels of nutrients thought to be available were actually not and the deer could do no better than first observed.

Including published digestibility values into this study has not been ideal as assumptions can only be made as to their impact on nutritive value. From the literature regarding digestibility there is a high possibility that the nutrient values have been overestimated despite taking care to minimise this. However Bissel (1958), working with white-tailed deer, believes that the quality of deer diets are seriously underestimated. By analysing preferred forage (as in the current study) and also rumen contents after 'free' feeding he found that the deer selected vegetation two to three times higher in protein than assumed. He concluded that preliminary evidence indicates that deer are capable of selecting plant material higher in protein than first supposed. This raises many questions but if it is the case then the overestimations thought to have been made in this current study may not be of any consequence as the actual diet the deer took could have been much higher in nutritive value if Bissel's (1958) findings are true.

The correlation data suggested that in most cases the availability of vegetation played a key role in the diets selected by fallow and roe deer. The data expressed as triangular diagrams to look at nutrient requirements also suggested that availability was heavily linked in. Tixier *et al* (1997) also suggested that the use of different plant species by roe deer was influenced by their availability. At each site, of the three most commonly eaten plant species in summer and winter, usually at least two were also in the top five most available in the local environment. Therefore, in winter particularly, the most available foods alone appear to have the necessary levels of nutrients to maintain the deer. This suggests that it may be coincidental that the deer fulfilled their needs: eating what was most readily available. The next step is to test whether or not fallow and roe deer can tell the difference between diets of different nutrient composition, this will be addressed in Chapter 6.

CHAPTER 6 DIET SELECTION USING FEEDING TRIALS

ABSTRACT

Feeding trials were carried out using captive groups of fallow and roe deer in parks across the South of England. Three trials with fallow deer and two trials with roe deer were performed using choice (preference) tests and diets that varied only in crude protein (%CP) content. The trials were designed to examine whether fallow and roe deer can select specific foods based upon nutrient content. In each trial the deer were shown to discriminate between the two foods of varying protein content when presented at the same time. Learning was strongly indicated because the deer switched from one food to the other generally after the first day, this suggested that post-ingestive feedback mechanisms were operating. The majority of the fallow deer populations arrived at a similar final preference scores (protein intake) in Trials One and Three, which translated into: Trial One 14%CP; Trial Three: 16%CP. Trial Two was inconclusive. The roe deer also showed similar preference scores at the end of Trials One and Two, but took a higher protein diet in each case (Trial One: 16%CP; Trial Two:19%CP). The trials suggested that protein content may be the basis for the selection of specific diets. Published crude protein requirements were much lower than the levels taken by both species of deer. The reason for taking excess may simply have been due to availability.

6.1 INTRODUCTION

The previous chapter suggested that fallow and roe deer do appear to select different diets at different times of the year based upon their nutritional content, this chapter aims to test this and investigate diet selection from an experimental perspective. This was done by conducting a series of feeding trials differing in one particular nutrient, protein. Protein was chosen as the main nutrient to investigate diet selection as it has been suggested by Parfitt (in Putman, 1986a) that it is a limiting factor in the diet of deer, particularly over the winter. The overall aim of the trials was similar to that of Chapter 5 but questioned the ability of the deer to select specific foods based upon the nutrient content.

Researchers have looked at diet selection by deer with reference to particular nutrients in their natural habitats, but very little has been done experimentally (*e.g.* cafeteria and pen trials with black-tailed deer: Gillingham and Bunnel, 1989; white-tailed deer: Berteaux *et al*, 1998). Controlled feeding trials have never been carried out on fallow and roe deer before. The first trial asked two questions concerning the discrimination abilities of these deer:-

1) **"can fallow and roe deer discriminate between two foods presented simultaneously based on their nutritional (protein) content?"**

2) **"can fallow and roe deer discriminate between two foods presented one at a time based on their nutritional (protein) content?"**

Using the results from the first feeding experiment subsequent trials were designed to further investigate the first question (simultaneous presentation), and in addition to answer the following question,

3) **"do fallow and roe deer take only their optimum protein diet when faced with a choice between this and a much higher protein diet?"**

The feeding trials were carried out using a preference test procedure conceptually similar to those used extensively for testing rats (*Rattus norvegicus*; *e.g.* Baker and Booth, 1989a) and also cats (*Felis catus*; *e.g.* Hegsted *et al*, 1956). An important factor in preference tests is that the foods taste or smell differently to allow the animals being tested to distinguish them. In most cases diets are made up so that one of them is more attractive (*e.g.* more palatable) and this is not the one the animals are expected to eventually choose. In general terms the aim of the preference test is to 'force' the animal to take the more attractive food first, thus enabling the researcher to see a switch to the other food or, as in most cases, for a mixture to be taken. In situations where the diets are very similar and there are no distinguishing sensory stimuli, researchers have used artificial flavours allowing the animals to associate particular qualities of the food with a particular flavour.

Deer are known to have a 'sweet tooth' (Goatcher and Church, 1970; Mason *et al*, 1993) and to choose foods with high sugar content (Bean and Mason, 1995). For the feeding trials in this study one of the diets contained more molasses than the other. Thus it was anticipated that the deer would take the diet with the molasses first and then over the following few hours/days would evaluate the food through post-ingestive feedback mechanisms (Provenza *et al*, 1994; Provenza 1995). This would, in theory, provide information that the molassed diet was inadequate in some form, encouraging them to switch to the other diet, either completely or as a mixture. It was not known how long the deer would take to switch from one diet to the next so the trials were run until the preference scores stabilised, ten days were forecast.

Two different preference test methods were used to answer the questions, one was a straightforward **choice test** seen in many preference studies (*e.g.* Baker and Booth, 1989a). The other tested the ability of the deer to discriminate between foods presented one at a time using a mixture of **choice tests and training days**, the latter allowing the animal to investigate each diet separately.

In the laboratory, preference tests are relatively easy to perform: an animal is presented with twice the weight of its normal intake of food divided equally between the two test diets. The animal is allowed to eat half of the total amount of food, *i.e.* its normal food ration. The remaining food is collected, weighed and the intake of each diet derived (Baker and Booth, 1989a), from these a preference score (Section 6.2.3.3) can then be calculated. Incorporating training days into this protocol simply means giving the animal their daily quota of food of each of the test diets on alternate days.

Researchers looking at preferences of deer have used cafeteria trials (*e.g.* Gillingham and Bunnell, 1989; see van Soest, 1994). These involve the deer being fed the test diet *ad libitum* and their behaviour monitored and in some cases the weight of the food eaten being calculated (Gillingham and Bunnell, 1989).

In this field study it would not be possible to stop the preference test when half of the food had been eaten as this could not easily be assessed, nor would it be possible to collect up and weigh what remained, as the diets were placed directly on the ground (normal for the deer). The deer would come to feed at the same time and probably, due to social influences they would feed at different rates. Thus a different method of measuring preference had to be devised (Section 6.2.3).

There have been very few studies investigating the protein requirements of fallow and roe deer, however, several studies have looked at white-tailed deer fawns and yearlings. The optimum protein value for these young, growing deer is between 13-16%CP (French *et al*, 1956). Smith *et al* (1975) provide evidence that 25% crude protein (CP) was required by fawns for maximum growth. Murphy and Coates (1966), showed that productivity, survival and condition of breeding females was adversely affected when CP levels were between 7 and 11%. They also found that the body weight and antler growth of yearlings and adult males was severely retarded at a level of 7%. Dietz (1965) also confirms this low protein level and claims that the function of the rumen is seriously impaired at less than 6-7%CP.

Reinken *et al* (1990) have included data on protein requirements of fallow deer in their discussion of techniques used in German deer farming. They quote seemingly very low winter requirements ranging from 6-10%CP depending on age, sex and body weight. These figures are very different to those presented for white-tailed deer (13-16%CP), similar in size to fallow deer. Red deer are reported to have protein requirements over the year ranging from 10% to 17% for females, 10-12% for males and 10-17% for calves (Adam, 1988), the lowest requirement is in winter. The winter crude protein levels of forage eaten by wapiti (or elk; *Cervus elaphus canadensis*), the North American sub-species of the red deer, also gave low levels, 4.9-5.8%, (Hobbs *et al*, 1981) suggesting their requirement is as low as suggested by Reinken *et al* (1990). Wallmo *et al* (1977), also analysed winter forage, but for mule deer, they found that in early winter the level was 7% which then dropped to 5% in late winter. These findings clearly indicate that a crude protein value of *circa* 6% is what most deer appear to be ingesting over the winter months, which is the point at which Dietz (1965) stated rumen function would be severely impaired.

When conducting preference tests the handling time, *i.e.* the time it takes the animal to pick up the food in its mouth, chew it and swallow it, must be considered for both diets. If there is a difference between the two and, for example, one food is easier to pick up or chew than the other then this could affect the final preference score as there could be a bias. Therefore handling trials were incorporated into the trials to validate the preference scores.

6.2 METHODOLOGY

The feeding trials were carried out using enclosed deer, over three winter field seasons Trial One in early 1991; Trial Two in early 1992; Trial Three late 1996 - early 1997. The trials took place during the winter months as this was the time when all the deer were accustomed to supplementary feeding of concentrate foods, generally pellets bought from commercial animal feed suppliers. Most were therefore used to coming to one particular area for feeding, at a particular time of day and also accepted the relative close proximity of people. Winter was also chosen because, as stated earlier, Parfitt (in Putman, 1986a) suggests that protein is a limiting factor in the diet of deer over the winter.

This section describes the study sites used (Section 6.2.1) and the diets (Section 6.2.2) for the feeding trials. It then considers the experimental protocol (Section 6.2.3) for fallow (Section 6.2.3.1) and roe deer (Section 6.2.3.2) and how preferences are recorded (Section 6.2.3.3). Handling trials are described in Section 6.2.4 and finally the trials (Section 6.2.5).

6.2.1 Study sites

Three fallow sites were used for Trials One and Two: Stonor Park, Henley-on-Thames, Oxfordshire; Magdalen College, Oxford; and Paultons Park, Ower, Hampshire. These three sites were used for Trial Three but in addition two further fallow deer groups became available at New Forest Nature Quest, Ashurst, Hampshire; and White House Farm, Easingwold, North Yorkshire. As mentioned all the parks were enclosed but varied in their size and holding capacity, from 3 (White House Farm) to 400 animals (Stonor, a subgroup of which were used in the trials).

An attempt was made to find enclosed roe sites, however roe deer are notoriously difficult to keep as they naturally occur singularly or in very small groups of two to four individuals (R.J. Putman, pers. comm.). The males also become dangerous to handle when they grow their first set of antlers (R.J. Putman, pers. comm.). This species was therefore poorly represented in the feeding trials. One group was used at Alice Holt Forestry Commission, near Wrecclesham, Alton, Hampshire and three individuals were used that had been hand reared separately at three places across southern England.

Stonor Park (OS grid reference SU744892)
Stonor Park was the largest park in this study consisting of grassland, deciduous and coniferous woodland. It contained between 200 and 400 fallow deer, during the three trials. The deer in this park had divided naturally into separate groups, one consisted of females, young and a few males. The other was predominantly male of varying ages with some females and numbered between 30 and 60 animals over the different trials. These two groups often broke up into smaller units of only a few animals, but came together when disturbed or fed.

The total number of deer in the park was too large for them to all take part in the feeding trials so as the deer were divided naturally into groups it was decided that the smaller should be used. Usually over the winter the two groups were fed together, however for the purpose of this study the smaller group was fed separately for a number of weeks prior to the trial, to accustom the deer to the new regime.

Magdalen College (SP520065)

The fallow deer at Magdalen College overwintered in a walled enclosure that was part of the main college. In the summer they were driven across the river into pastures bordering the college grounds. Over the study period this group consisted of 5-7 males, 22-23 females and 12-13 fawns. They were all fed together and as it was not possible to divide them into smaller groups the entire group was studied for the trial.

Paultons Park (SU312166)

The fallow deer at Paultons Park were kept in a comparatively small enclosure. In Trial One the group comprised two males, one of which (according to the keepers and observed rutting behaviour) appeared dominant over the other, five females and two fawns. The group was reduced in Trial Two, the two female fawns from Trial One were present as adults but four of the original females had either been sold or had died. Trial Three had eight deer: one male; four females and three fawns (none of these were thought to have been in Trials One or Two). These deer were usually fed concentrates and provided with hay all year round as there was not enough forage in their enclosure to sustain them.

New Forest Nature Quest (SU354098)

These fallow deer were enclosed in a large paddock and consisted of three males, seven females and three fawns. Unfortunately they shared their enclosure with approximately 18 Soay sheep and also two red deer males. The deer were fed all year round and were accustomed to having their feed laid out in lines. For the duration of the trial the two red deer and the sheep could not be relocated, however the red deer were removed prior to feeding and then put back afterwards, the sheep were left to feed with the fallow deer. The keeper believed that they would not affect the deer's selection of feeds as the deer were clearly dominant over the sheep, but this was not the case for the male red deer, hence their removal (Derek Gow, pers. comm.).

White House Farm (SE509709)

Three captive reared fallow deer (two females; one male), were kept in paddock on a working farm, all the animals were young (approximately 20 months old) and it was suspected that the two females were pregnant (this was later proved correct by the subsequent production of one fawn each). These deer were normally fed concentrates on the ground.

Alice Holt (SU805419)
The Forestry Commission at Alice Holt have a well established enclosure containing three roe deer; a male and two females (one of which was the male's mother). The deer were often fed concentrates in the winter from a covered food hopper (Figure 6.1). Because this hopper was large enough to allow all three animals to feed at the same time, it was decided that it should be used for the trial food. Prior to the trial, the keeper inserted a division so that the two test foods could be placed either side.

Individual roe
Three individual roe deer also participated in the feeding trials. All of these animals were handreared, either having been injured as fawns or been picked up and handed in by the general public mistakenly thinking they had been abandoned. One yearling female (at the time of Trial One) was kept in a small garden enclosure in Burley in the New Forest (SU205043), and two very young males (less than 1 year old) were kept at separate households, one in a small garden enclosure in Hursley Hampshire (SU418235), the other in a similar enclosure on the Gatton House Estate, Reigate, Surrey (TQ275529).

Apart from the Forestry Commission's roe deer which were normally used for a variety of trials, all the deer (fallow and roe) were kept for recreational or aesthetic purposes. Those at Stonor were in the grounds of a stately home similarly the fallow at Magdalen were kept in the grounds of the college. Paultons Park and Nature Quest were parks open to the public, however at the times of the trials they were closed. The deer at White House Farm were for the enjoyment of visitors to the farm's holiday cottages. None of the animals at any site were kept for farming purposes although at Stonor and Magdalen several were culled annually to regulate numbers and provide venison locally.

6.2.2 Diets

When carrying out a choice test where one specific component is being investigated (*e.g.* protein), it is very important to standardise all other variables (*e.g.* dietary properties) in the two diets. This eliminates the risk of animals selecting on the basis of other factors. In this study protein selection was being investigated. Therefore, the diets contained different levels of protein and as far as possible this was all that was different. One factor that could have influenced selection (other than protein) was the metabolizable energy (ME) content of the diets, so attempts were made throughout to keep the ME content of the paired diets as similar as possible.

Figure 6.1 Feeding trough used by roe deer at Alice Holt for the feeding trials. (partition is not present in the photograph but was included in the trials)

Original recipes for five of the six diets for Trials One-Three (Appendix 11) were devised by myself with the aid of published tables (McDonald *et al,* 1981; van Soest, 1994; BOCM PAULS, 1996). These were then manufactured by the Rowett Research Services Limited (RRS) of Aberdeen, Scotland. The nutritional staff at the RRS checked the recipes and calculations, and amended these to incorporate minerals and vitamins (dicalcium phosphate, calcium carbonate, magnesium oxide, sodium chloride and vitamin supplements as used for domestic livestock such as lambs). The crude protein content (%CP) of each diet will be discussed for each trial in turn (Sections 6.3.1, 6.4.1, 6.5.1).

The feeds were made in the standard pelletted format commonly used in commercial deer feed (*e.g.* Dukes Deer Choice 18 Nuts). The diets were put in 25kg paper sacks and on arrival in Southampton, each diet was stored in a cool, dark and dry place. The feed could be kept in this way for 4-6 months before the added vitamin supplements would start to degrade (Rowett Research Services, pers. comm.) however, it was generally used within 2-3 months of arrival. The keepers were consulted as to how much food the deer were usually given per day. As a result fallow deer were offered 0.7-0.8kg/deer/day and roe deer 0.33-0.5kg/deer/day of each test diet, this was twice their normal daily requirement as per usual laboratory based preference tests.

To minimise expenditure one of the diets used in Trial Three (Table A11.6, Appendix 11) was a commercially made deer feed, purchased from Duke's of Botley Agriculture Ltd, Hampshire. The recipe was obtained from the company's nutritionists and a contrasting diet of lower protein content was made up using as many of the same components as possible (Table A11.6, Appendix 11). These were chosen by the RRS based upon what ingredients they had in stock and what their machines were capable of processing.

As stated earlier, to demonstrate a preference experimentally, the diets have to be different from one another in at least one sensory stimulus (Section 6.1). In this study one of the diets in each pair was made sweeter than the other. In order to confirm this, each diet was tasted by a human shortly after its arrival, and differences could be detected. The smell of diets was also noted and olfactory differences could be detected, although for the diets in Trial Two this was less apparent (Section 6.4). It was assumed that if a human could detect differences in the pairs of diets being used, then deer would certainly be able to, as olfaction has been shown to be an important sense used by ruminants (sheep: Tribe, 1949).

6.2.3 Test protocol

6.2.3.1 Fallow deer

For the **choice tests** (including those with the training days) the diets were placed on the ground in lines from a video camera (*Trial One*: Panasonic NV-M7B; *Trial Two*: Phillips Explorer VKR6850; *Trial Three*: Sony Video 8 CCD-TR37OE) that was partially concealed in a building or behind a fence (Figure 6.2a). The lines were positioned at least 2m apart and were long enough (up to 12m) and in sufficient numbers to allow all the deer to feed where they wanted with minimal social disturbance (Figure 6.2b). The order in which each diet was laid out was alternated daily to eliminate any bias towards a particular part of the enclosure, *e.g.* furthest from the fence.

All of the fallow deer were accustomed to being fed concentrates at the time of the trials and they were normally fed on the ground between 8 and 9 a.m. For one week prior (Stonor three weeks prior) to the trial commencing the keepers were asked to place their normal food in lines representative of the test situation, and in the area where the test would take place if this was not their usual feeding area (Stonor).

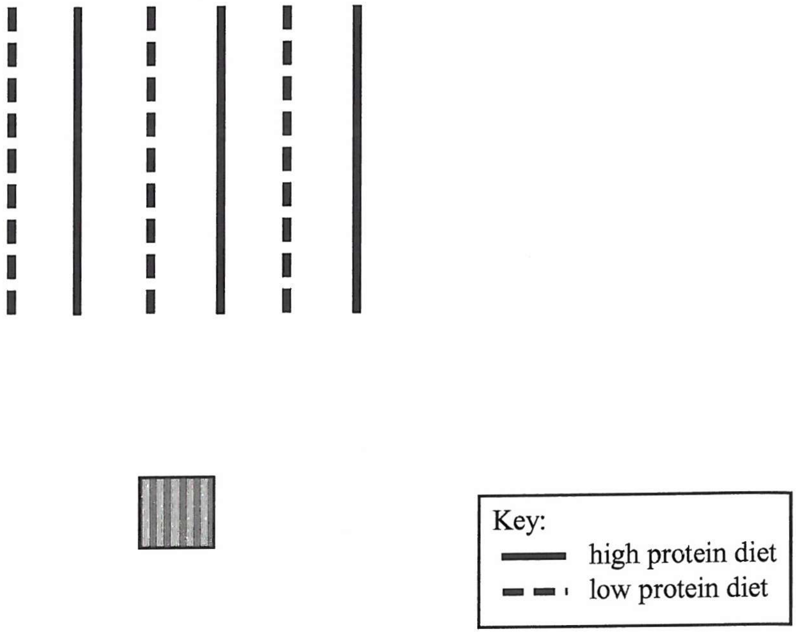
At the beginning of each trial day the keeper was instructed as to the order in which the food should be laid out (*e.g.* diet 1, diet 2, diet 1, diet 2). Attempts were made throughout the trials to keep conditions as near to the usual routine as possible. This reduced the risk of invalid data through the deer being disturbed and nervous, hence the keeper (a familiar individual) put out the food at the usual time and place. Once the feed had been laid out the keeper retreated from the feeding area/enclosure and the video camera was set to record by the experimenter, who remained out of sight. The deer were left to feed for as long as they wanted, and when all the deer had left the lines of feed, recording was terminated. The food was left on the ground and in all cases was consumed by the time of the following day's test. At all of the parks except Stonor, the experimental subjects were the only deer which could have eaten the remaining food, however grey squirrels (*Sciurus carolinensis*) and crows (*Corvus corone*) were also observed eating each of the test diets.

The choice tests were continued for 10-11 days, by which time the preference scores stabilised. The scores (Section 6.2.3.3) were calculated each test day to monitor the progress.

The protocol for the preference test using training days is displayed in Table 6.1. Choice tests (as above) were performed on the first two days, to get a baseline preference score, followed by

Figure 6.2 Method of recording feeding trials and placement of diets.

a) Alternating lines of feed (solid and broken lines) of the two diets, radiating out from the video camera (square).



b) Feeding trial at Stonor Park showing the alternating lines of feed with sufficient length and space between to accommodate all the deer in this group

two alternating training days (*e.g.* diet 1, diet 2), where one food was laid out in the same way as for the choice tests (Figure 6.2a) but was not videotaped. A choice test was done on Day 5 to monitor progress followed by four more training days (alternating the diets) and two more choice test days to get the final preference scores (11 days total).

Table 6.1 Order of choice tests and training days.

Day										
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
choice test	**choice test**	training Diet 1	training Diet 2	**choice test**	training Diet 1	training Diet 2	training Diet 1	training Diet 2	**choice test**	**choice test**

6.2.3.2 Roe deer

Choice tests were used for the roe deer. As mentioned, the deer at Alice Holt were accustomed to feeding out of a trough and so in order to retain normality the trough was used for the trials. It was divided using a wooden board (Figure 6.1) allowing the two diets to be presented simultaneously without mixing. The side of the trough into which each diet was placed was alternated to eliminate the possibility of any side bias. The individual roe deer were also fed in their usual way either on the ground or in their food bowls.

6.2.3.3 Recording preference scores

From the video recordings, scans of each line were made at one minute intervals and the number of deer feeding on each line was recorded. A preference score was derived for each study group (population) each day. The preference scores were expressed as a preference for one of the two protein diets being tested and were calculated as follows:-

$$\frac{\text{number of deer present on X protein diet}}{\text{number of deer present on X protein diet} + \text{number of deer present on Y protein diet}}$$

Population preference scores were calculated for the deer at each of the parks. In addition, when it was possible to identify and observe individuals over the entire test period (Paultons Park, White House Farm and Alice Holt), the actual time each individual spent feeding (minutes) on each diet was recorded (continuous sampling). Therefore, for these animals more accurate preference scores could be determined for the whole group. These individual preference scores were calculated in the same way by replacing number of deer with actual time spent feeding.

6.2.4 Food handling trials

To obtain handling rates, an animal is given a known amount of food which they are left to eat. The time taken to eat is recorded and any uneaten food is collected and weighed. The total intake is calculated and using the total feeding duration, a rate of intake (g/min) is derived for the given food. In this study only the parks with small numbers of deer could be used as individuals had to be observed eating, also to be able to the weigh the uneaten food it had to be placed in retrievable dishes. Sufficient dishes were used so that each deer in the group could have its own dish. These were arranged on the ground in an array aimed at encouraging the deer to stay at one dish throughout (Figure 6.3). A small amount of food (300g) was placed in each dish and the deer were video taped eating. If any food was left (after the deer had left) it was noted which dish it came from and was collected and weighed. The video tape was observed and the feeding duration of each animal that remained solely at one dish was recorded. This duration was then used to calculate a handling rate (g/min) for each animal for each food and a mean was then taken.

Handling trials were carried out after the main preference trial had ended. Each trial ran over four days and was performed at the usual feeding time. On each day a different test diet was given such that each was given twice, alternating over the days.

Unless an animal is in close captivity and/or can be observed very closely, it is very difficult to record the time spent chewing and finally swallowing. When observing animals from a distance, certain assumptions have to be made, it is possible to see an animal pick up food and then it can be assumed that it has chewed and swallowed that mouthful when it either goes to eat more or moves away from the feeding area. These assumptions were made in the case of the handling trials used here.

Handling trials were only performed using the fallow deer, this was due mainly to the ease of access

Figure 6.3 Handling trial showing how the deer were encouraged to eat from one dish only.

but also to the secretive nature of the roe at Alice Holt. For the diets in Trial One, the deer at Paultons Park were used in Handling Trial One. Due to the lack of success with the diets of Trial Two no handling trial was conducted. A handling trial for the diets of Trial Three was carried out on the deer at Paultons Park and also the three deer at White House Farm.

6.2.5 The trials

The results from the first trial determined the procedure and diet for the second and third trials therefore the method and results for each trial are described sequentially, Trial One (Section 6.3), Trial Two (Section 6.4) and Trial Three (Section 6.5). The pairs of diets tested against each other in the three feeding trials are shown in Table 6.2. the reasons for the choice of protein levels will be discussed for each trial.

The theory behind all the feeding trials was that the deer would initially take one protein diet over the other because it contained molasses making the food sweeter and more palatable. Over the following days the deer would evaluate the food through post-ingestive mechanisms (Provenza *et al*, 1994; Provenza, 1995) providing information that the diet was incorrect, therefore forcing a switch to be made to the other protein diet.

Where possible each results section will include measurements of factors that could affect the preference scores, *i.e.* handling time, number of feeding bouts and use of the feeding lines.

Table 6.2 The protein content of the three pairs of diets tested against each other in each of the three feeding trials (LP = low protein diet, HP = high protein diet, OP = optimum protein diet and the numbers 1,2 and 3 refer to the trials).

	Protein levels (% crude protein)	**Codes for protein levels**
Trial One:	9.35 vs 15.71	LP1vs HP1
Trial Two:	**13.65** vs 26.88	OP2 vs HP2
Trial Three:	**13.54** vs 21.89	OP3 vs HP3

6.2.6 Data preparation and statistical analysis

The preference data for all trials were transformed using arcsine square root transformation acceptable for data recorded as proportions (Dytham, 1999). Where parametric tests were used on data other than the preference scores they were transformed using a log transformation ($\log_{10}$). Non-parametric and parametric tests were used to test the data (see Section 2.2.5.6 for assumptions and data requirements). All hypotheses were tested using a critical probability (P) value of 0.05, unless otherwise stated. The following descriptions of each analysis is based on SPSS Base 7.0 for Windows, User's Guide (1996), Fowler *et al* (1998) and Dytham (1999).

Levene test: see Section 4.2.8.3

Independent t-test: Compares means for two independent groups of data. The test assumes that the data are continuous, approximately normally distributed with homogeneous variances. This latter assumption is tested using the Levene test (see Section 4.2.8.3).

One sampled t-test: Tests whether the mean of a single variable differs from a specified constant, in this study the constant was a preference score of 0.5 (or 0.785 when transformed using arcsine square root).

Analysis of Variance (ANOVA): see Section 2.2.5.6

Kruskal Wallis test: see Section 2.2.5.6

Mann Whitney test: see Section 2.2.5.6

6.3 TRIAL ONE

6.3.1 Method - Trial One

This trial was exploratory, firstly to investigate whether the deer had the ability to learn crude protein content of different diets and secondly to find the levels they would select if they did indeed have the ability. Upper and lower limits were derived from the published data discussed in Section 6.1.

Ingredients, *e.g.* hay, flaked maize, molasses and soya bean meal, known to be palatable to deer (Rowett Research Institute pers. comm.) were used to make up the two diets for Trial One. From recorded values of the CP and ME (McDonald *et al* 1981; van Soest, 1994; BOCM PAULS, 1996) recipes for two diets were formulated. This was done by altering the proportions of the ingredients until the required protein level was reached (Appendix 11). The two diets for this trial were 9.35%CP (LP1) and 15.71%CP (HP1) and were manufactured to the set specifications by Rowett Research Services Ltd. (RRS). These protein levels were appropriate as the low value was not lower than the recorded minimum value of 7%CP (Murphy and Coates, 1966) and the high value fell inside the optimum range (French *et al*, 1956). As Table A11.1 in Appendix 11 shows LP1 contained more molasses than HP1, this is sweet and was altered to encourage the deer to take the lower protein diet first and then switch to the one within the deer's optimum range.

The fallow deer at Paultons Park and Magdalen College, the individual roe deer and those at Alice Holt were used to answer the question "can deer learn the nutritional content (protein) of two foods presented simultaneously?" and were preference tested every day (continuous testing). The fallow deer at Stonor Park were used to answer "can deer learn the nutritional content (protein) of two foods presented one at a time?" using preference tests and training days. Stonor was used for this question as this park was difficult for the experimenter to get to every day and the keeper could conduct the training days himself.

6.3.2 Results - Trial One

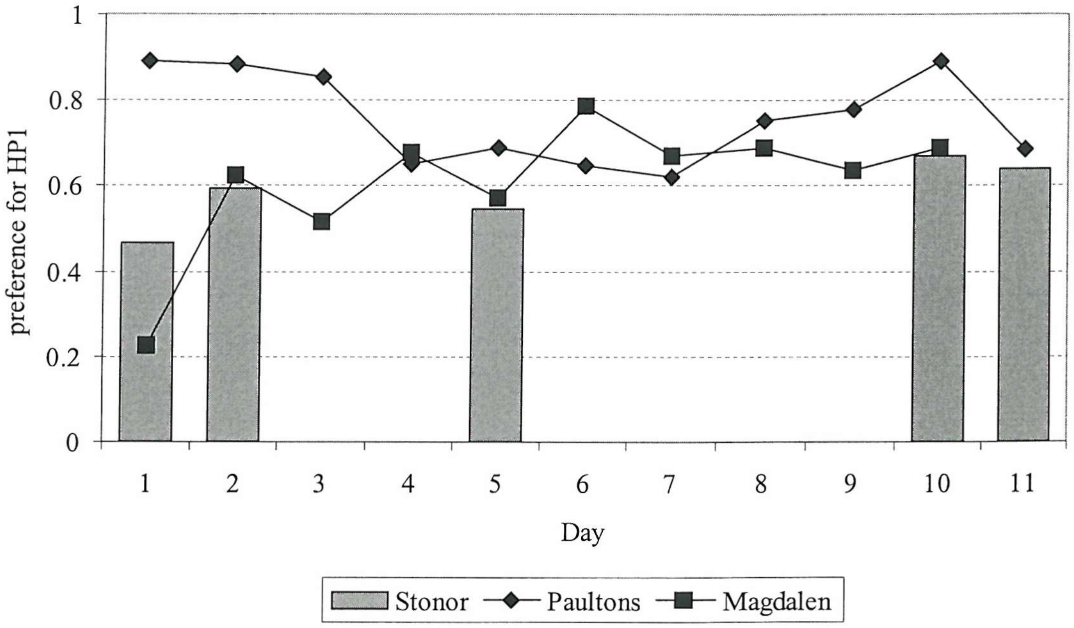
6.3.2.1 Fallow deer

Population preferences

The population results for the fallow deer were taken from the scanned recordings of the number of deer on each line of food at one minute intervals. The length of time the deer spent feeding varied from day to day (Table A12.1, Appendix 12). The length of time over which the preference ratings were determined was standardised. On most trial days the deer at all three parks fed for at least 30 minutes, hence this was taken as the cut-off and preference scores for each day were calculated on this basis. Where the deer fed for less than 30 minutes (Table A12.1, Appendix 12) the preference scores were calculated over the total time.

Figure 6.4 shows the preferences scores for the HP1 diet of the fallow deer at Paultons, Magdalen and Stonor. The results for Magdalen and Stonor follow a similar trend in that the deer begin by selecting largely the LP1 (Magdalen = 0.227 and Stonor = 0.466 preference scores for HP1). Over the following days, whether this involved continuous preference testing or training, both parks stabilised at a similar preference level (Magdalen = 0.688; Stonor = 0.64). This overall trend was not true at Paultons. The deer selected HP1 most readily at the beginning of the trial (0.89); after the third day their preference decreased arriving at 0.683 on the last day. The tenth day at Paultons should be noted separately as there was a lot of disturbance outside the enclosure and this almost certainly accounted for the increase in preference for HP1 on that day.

Figure 6.4 Preference for HP1 by the fallow deer at Paultons Park, Magdalen College (continuous testing) and Stonor Park (testing days represented as bars + no data were recorded on training days).



The final preference scores at the three sites were tested to show that there was statistically a preference for one food over the other (in this case HP1 over LP1). For this to be the case the data have to be significantly different to 0.5, *i.e.* no preference. A one sampled t-test was used on the arcsine square root transformed preferences, acceptable for proportion data (Dytham, 1999), which tested the following hypothesis:-

H_0 = no preference was shown for one food over the other

The t-test gave a significant difference ($t_2 = 10.702$, $P<0.01$), the null hypothesis was rejected. The preference scores for the three sites showed that fallow deer did indeed have a preference and that it was for HP1 in favour of LP1.

As the aim of this feeding trial was to look at the selection of protein, the final preference scores achieved by all three parks were converted into % crude protein (CP) eaten. The deer at Paultons took a diet consisting of 13.69%CP (HP1: 0.683 x 15.71%CP + LP1: 0.317 x 9.35%CP); Magdalen took 13.73%CP (HP1: 0.688 x 15.71%CP + LP1: 0.312 x 9.35%CP); Stonor took 13.42%CP (HP1: 0.64 x 15.71%CP + LP1: 0.36 x 9.35%CP). The mean from these data suggested that fallow deer prefer to take a mixed diet of ***approximately 13.6%CP*** when presented with a choice.

Scans vs. continuous sampling (actual time spent feeding)

More detailed recordings of the actual time spent (minutes) on each line of food was carried out for the deer at Paultons. Individual preferences (except for females 4 and 5, where their preferences were averaged due to an inability to identify each animal consistently over the 11 days) were recorded and a mean preference calculated. In order to test the observation methods used, the means derived from continuous sampling were compared to the data from the scanning method. The data were transformed using arcsine square root transformation. A one-way ANOVA was then performed to examine the effect of the type of observation method used. The null hypothesis being tested was:-

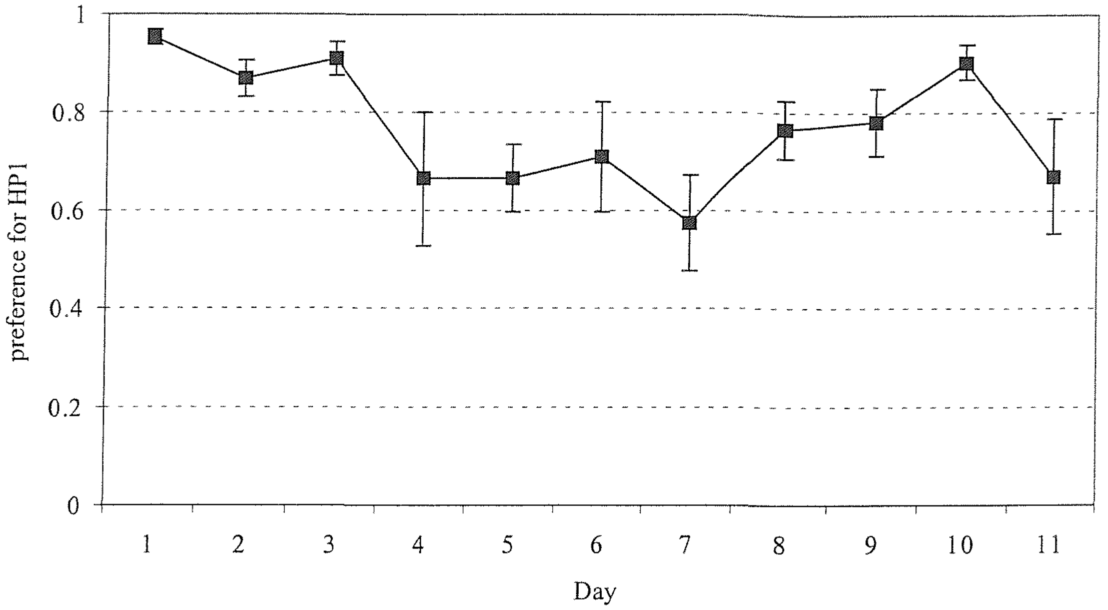
H_0 = there was no difference in the preferences scores obtained using the scanning or continuous sampling methods

No significant difference was found ($F_{1,131} = 0.692$, NS) between the two observation methods, therefore the null hypothesis was accepted. This indicated that either method was acceptable when measuring the preference for HP1 at Paultons Park. This study assumes that this can be extended to the other sites and other trials, suggesting that the population values achieved were representative of the whole population as if each individual had been measured separately.

Individual preferences from continuous sampling

The means from the individual deer at Paultons are presented in Figure 6.5, the standard error bars indicate a considerable degree of variation within the group, especially after Day 3.

Figure 6.5 Mean preference for HP1 (± standard error), using the individual preference scores of the seven fallow deer at Paultons Park.



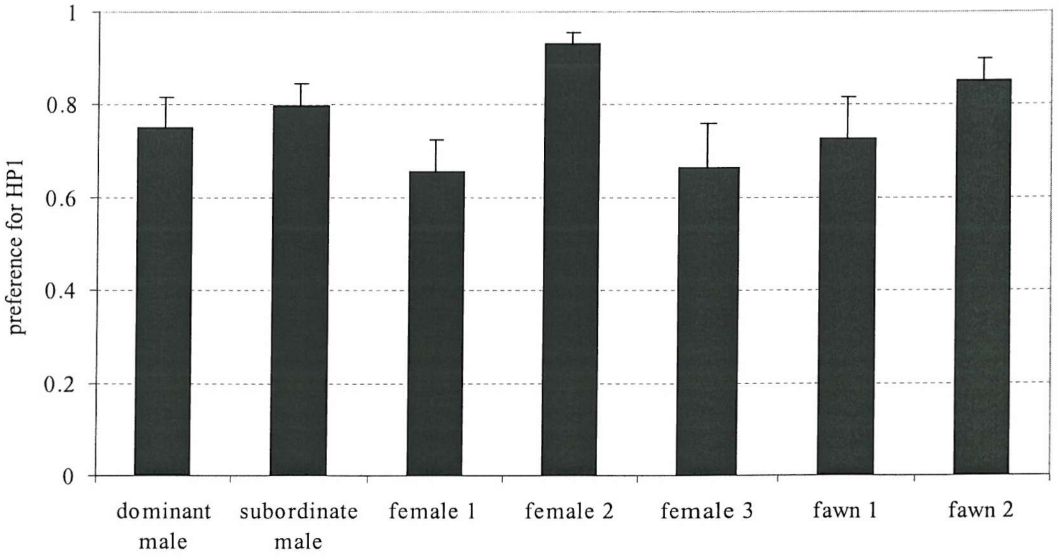
These data were explored further to investigate the preference scores of specific individuals but more importantly from a potential management point of view, the effects of age and sex upon the preference for HP1. The effect of the different days was also included in the model. ANOVA on the transformed data was used and the deer were divided into the following age/sex classes: Adult male = dominant male and subordinate male; Adult female = female1 and female 3; Yearling female = female 2; Fawn = fawn 1 and fawn 2. The ANOVA showed a significant effect of the age/sex class ($F_{3,63}$ = 5.363, $P<0.005$) where a post hoc Duncan multiple range test highlighted the young female as being significantly different from the other individuals, with the highest mean preference (0.931) for the HP1 diet (Table 6.3).

There was also a significant effect of day ($F_{10,63}$ = 2.871, $P=0.005$) where Day 7 showed the lowest overall preference for HP1 (0.591) and Day 1 showed the highest (0.963). The individual deer were investigated to see the extent to which each varied in their the preference for high protein. This also produced a significant result ($F_{7,60}$ = 3.069, $P<0.05$) where the multiple range test showed again that it was the young female that had the highest overall preference, and she was significantly different from female 1 in particular, but also female 3, one of the fawns and the dominant male (Figure 6.6).

Table 6.3 Multiple range (Duncan) test for the effect of **age/sex class** on the preference for HP1 (preference scores presented are back transformations of the means of the arcsine transformed data) means followed by the same letter are not significantly different from each other.

Age/sex class	**Mean value (preference score)**	**Protein intake (%CP)**
Young female	0.947 a	15.373
Fawns	0.834 b	14.654
Adult males	0.812 b	14.514
Adult females	0.685 b	13.707

Figure 6.6 Mean preference scores for HP1 (± standard error) over 11 days for each individual deer at Paultons Park (N=11).



As with the population final preference scores, those for the individual deer were tested to see if they deviated from no preference (score of 0.50) using a one sample t-test on the arcsine square root transformed data. The same null hypothesis was tested:-

H_0 = no preference was shown for one food over the other

The test produced a non significant result ($t_6 = 1.449$, NS), accepting the null hypothesis and suggesting there was no preference shown by the group as a whole.

6.3.2.2 Roe deer

Alice Holt

The preference test with the roe deer at Alice Holt was designed to look at Question 1 (can deer learn the nutritional content of two foods presented simultaneously?), however the procedure of recording the data differed from that used at Paultons and Magdalen. The enclosure the roe deer occupied was designed for the Forestry Commission to observe them 24 hours a day as they were used for a number of different trials. The diets were put out into their usual trough and the Forestry Commission's own video equipment was used. The data collected were not as conclusive as for the fallow, this may have been due to the different species of deer or more likely the lack of control over the filming.

There were three roe deer in the enclosure: an adult male and his mother and another adult female distinguishable by a collar around her neck. From observing the tapes it became apparent that the manner in which the deer fed at the trough was very irregular. The male and his mother generally fed together and the collared female fed alone. On some days the animals did not appear to feed or fed for very short periods, there was no obvious reason for this behaviour and the three individuals did not follow the same daily patterns. The preference scores for each animal were calculated using the continuous sampling method, as used for the deer at Paultons Park (Table 6.4).

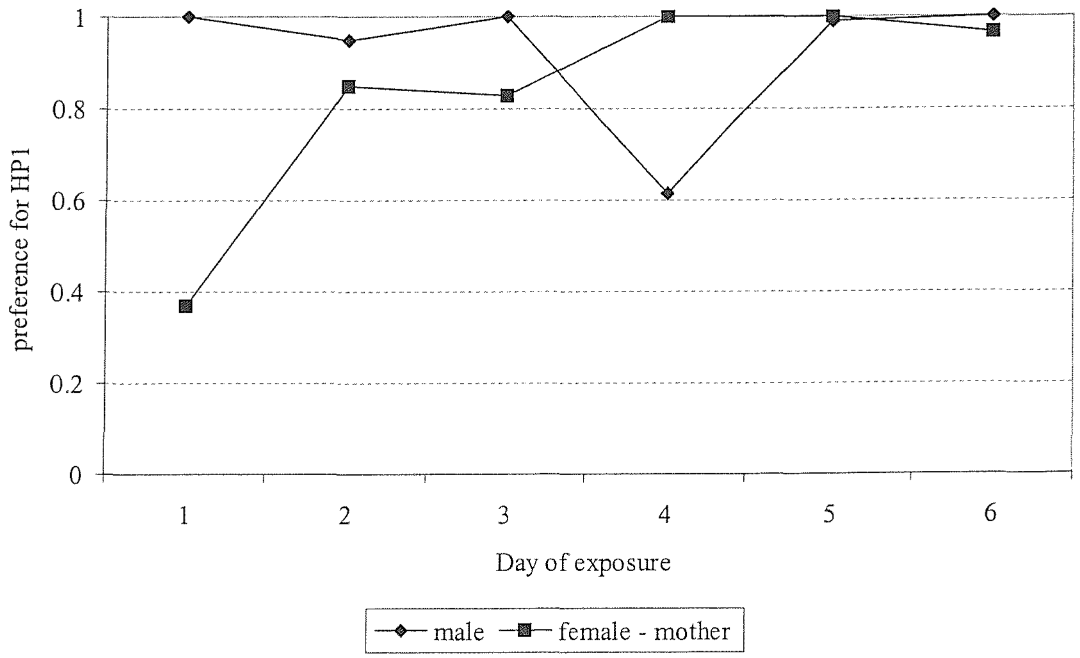
Days in which an individual's feeding time was less than one minute were omitted as they were thought to be incomplete, broken off for some reason, and not a true reflection of the preference for that day. As a result of these omissions there was not a complete set of data for each individual for the ten days of the trial. Therefore, instead of the preference scores being listed from day one of presentation, for the roe deer they are listed from day one of exposure, *e.g.* on the actual first day of presentation only the collared female came to the trough to feed, whereas on day two the male and his mother also came to feed, making this their first day of exposure and the collared female's second.

Table 6.4 Preference scores for the HP1 diet and the percentage crude protein intake (%CP) for the three roe deer at Alice Holt

Day of exposure	Adult Male		Female Mother		Female Collared	
	Preference score	%CP intake	Preference score	%CP intake	Preference score	%CP intake
1	1	15.71	0.368	11.69	0.011	9.42
2	0.95	15.39	0.848	14.74	0	9.35
3	1	15.71	0.826	14.6	0.097	9.97
4	0.613	13.23	1	15.71	1	15.71
5	0.991	15.65	1	15.71	1	15.71
6	1	15.71	0.966	15.49	0.462	12.29

From Table 6.4 it is clear that the collared female fed very differently to the male and his mother. The reason for this is not clear it may have resulted from social pressures for the other two deer. The male and his mother displayed more consistent preference score for the HP1 diet. If the data for the collared female are removed and that for the other two individuals plotted a pattern becomes more obvious (Figure 6.7).

Figure 6.7 Preference scores of the male roe deer and his mother.



On the first day of exposure the male had a preference score of one, *i.e.* he ate only HP1, after this his score remained high except for the fourth day of exposure when, for some unexplained reason it decreased to 0.613, but recovered afterwards. On the first exposure to the feed his mother ate similar amounts of each, but showed a marginal preference for LP1. After this her preference for the high protein increased and her scores reflected those of her son. The final mean protein intake was 15.6%CP.

Individual roe deer

Unfortunately all of the individual roe deer were reported by their owners to have eaten very little or none of each diet. It was believed that the usual diet of one of the deer, that was particularly tame and had been in captivity for some time, was too dissimilar from the format of the test diets that she would not eat either of them. This may also have been the reason for the other individuals not eating.

6.3.2.3 Factors affecting preference score

There are a number of factors that could potentially affect the preferences for the test diets. ***Handling time*** has already been discussed in Section 6.2.4 and was investigated using a separate protocol to the main preference test. The social hierarchy within the groups could also affect the preference scores, this was investigated from the individual trial data by looking at the number of ***feeding bouts*** performed by each of the deer. Finally, the ***use of the lines*** of feed could have had some influence over the foods that were eaten. This was also investigated from the individual trial data.

For reasons described in Section 6.2.4 the handling trial for the first two test diets was only carried out at Paultons. The social hierarchy and the use of the lines will also only be described here for the deer at Paultons.

Handling trial one

This handling trial was carried out at Paultons Park over four days alternating the test diets. Observation of the video tape showed only the two male deer fed individually from one dish each throughout the trial. Therefore data could only be used from these animals and there could be no representation of the females. Table 6.5 shows the handling times for the two male deer at Paultons Park.

Table 6.5 Handling times (rate of consumption: g/min) of the two test diets in Handling Trial One (HP1 = high protein diet, LP1 = low protein diet both from Trial One).

		Rate (g/min)	
Day	**Diet**	**Dominant Male**	**Subordinate male**
1	HP1	39.18	31.05
2	LP1	30.37	43.30
3	HP1	49.81	61.35
4	LP1	40.06	46.79

The data were analysed initially to investigate any differences between the two males. An independent samples t-test was used, but firstly the variances were tested for homogeneity, (an assumption of the test) using the Levene test. This produced a non significant result, allowing the t-test to be used to test the following null hypothesis:-

H_0 = the handling time of the two males was the same

The t-test produced a non significant result ($t_6 = 0.776$, NS) so the null hypothesis was accepted. Subsequently, the data from the two males were pooled to assess differences between the two diets. T-test was again used to test the following null hypothesis:-

H_0 = the handling time for both diets were the same

The t-test indicated no significant difference between the handling times of both diets ($t_6 = 0.698$, NS) and so the null hypothesis was accepted.

Feeding bouts

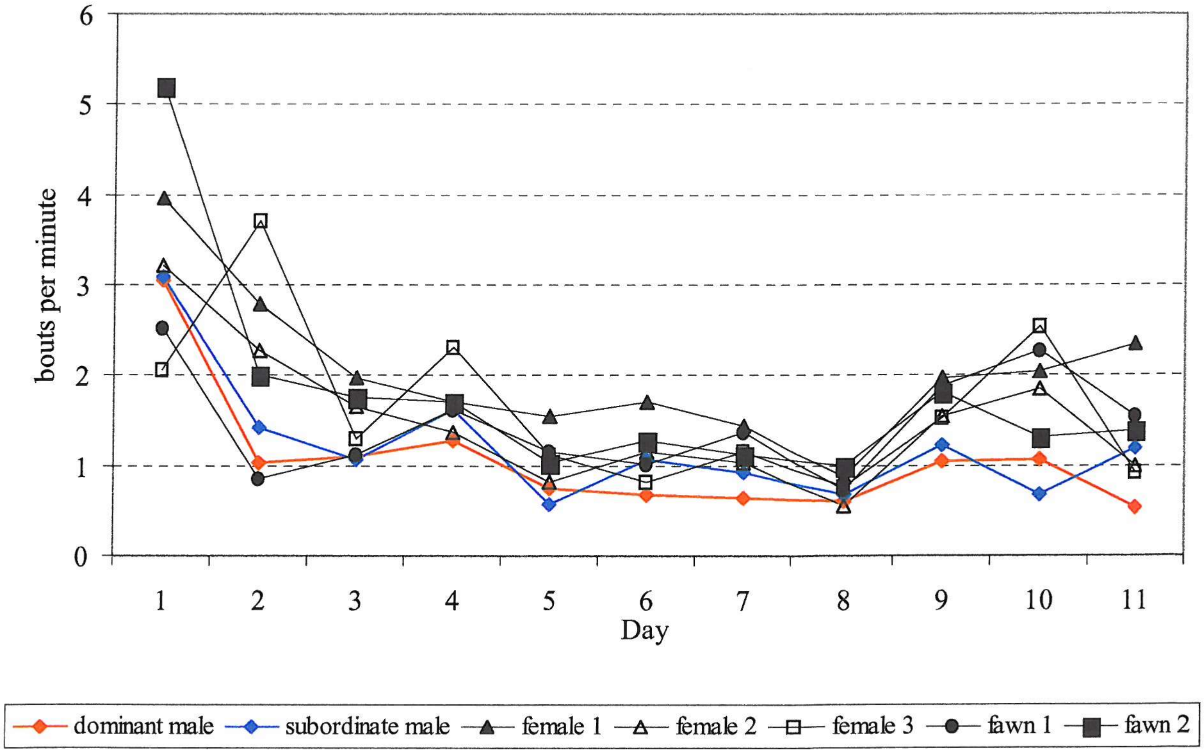
The number of feeding bouts performed by the deer (excluding females 4 and 5) at Paultons Park was also investigated to see if there were any differences between the individuals or over time. A feeding bout was defined as a discrete length of time spent feeding, which in this case could be terminated in a number of different ways, *i.e.* freely moving to another line or along a line or following pressure from another deer. Figure 6.8 shows the number of bouts performed per minute by each deer on each day. The red line indicates the dominant male who appeared to show the lowest number of feeding bouts per minute overall. The blue line is the subordinate male and follows the pattern of the dominant male extremely closely, only on two occasions does the subordinate perform fewer bouts per minute (Days 5 and 10).

This relationship between the individuals was analysed to look for effects of individuals and time (day). This was performed using ANOVA, however firstly the data were normalised by logarithmic transformation ($\log_{10}$). The following null hypotheses were tested:-

1. H_0 = each individual performed the same number of feeding bouts per minute
2. H_0 = the number of feeding bouts per minute were the same on each day

The ANOVA showed that there was a significant effect of the individual deer with respect to the number of feeding bouts performed per minute ($F_{6,60} = 8.348$, $P<0.001$), so the first null hypothesis was rejected. A multiple range test (Duncan) revealed that the dominant male was significantly

Figure 6.8 Number of feeding bouts per minute for each of the deer at Paultons Park, with the lines from the two males highlighted.



different to the rest of the group except for the subordinate male, as suggested in Figure 6.8. The groupings are shown on Table 6.6. Also there was a significant effect of day ($F_{10,60} = 15.305$, $P<0.001$), so the second null hypothesis was rejected. All the deer on Day 1 showed higher numbers of bouts per minute than on any other day. This could simply have been due to the novel food they had been presented with and also the presence of the camera outside their enclosure, increasing their vigilance so there were more bouts of shorter duration. After Day 1 all the deer (with the exception of female 3) decreased their frequency of bouts, in particular fawn 2.

Table 6.6 Multiple range (Duncan) test for the effect of **individual** on the number of feeding bouts performed per minute, showing the back transformed mean feeding bout length (means followed by the same letter are not significantly different from each other).

Deer	Mean value
Female 1	1.898 a
Fawn 2	1.578 a b
Female 3	1.464 b
Fawn 1	1.359 b c
Female 2	1.338 b c
Subordinate male	1.099 c d
Dominant male	1.059 d

Use of lines

The duration of time the deer spent on each line of feed was important to consider because if one line was favoured, the preference scores may have been affected. An attempt was made to minimise this risk by alternating the two diets between the lines every day. A Kruskal Wallis test was used to look at the following null hypothesis:-

H_0 = all four lines of feed were used to the same degree

The null hypothesis was accepted as the Kruskal Wallis test showed no significant difference between different lines with respect to their usage ($\chi^2_3 = 2.262$, NS).

6.4 TRIAL TWO

6.4.1 Method - Trial Two

Trial One suggested that there was a mean optimum protein level (13.6%CP), that the fallow deer preferred (Section 6.3.2.1). Therefore in an attempt to further test the learning and discrimination capabilities of the deer Trial Two compared a diet that approximated the optimum level, 13.65%CP (OP2) with a very high protein diet, 26.88%CP (HP2), the latter of which was made more attractive, again by adding molasses. In this case RRS were asked to make up two diets to the required protein specifications (Tables A11.3 - 4, Appendix 11).

Only choice tests were carried out with these diets. Trial Two was conducted at Paultons Park, Magdalen College, Stonor Park and Alice Holt.

There were a number of problems with this trial (see Section 6.4.2) so diets were flavoured to encourage consumption. Flavours known not to inhibit deer feeding are almond and peppermint (R.J. Putman, pers. comm.). The dilutions used were determined by human smell and taste both on initial application and after 30 minutes (in case the deer did not eat the food immediately). Almond was used at 50% dilution and peppermint at 35%. The deer at Paultons Park had OP2 + peppermint and HP2 + almond. At Stonor the flavours were reversed to check for preference of a particular flavour. The diets were placed in large buckets, sprayed, thoroughly mixed and laid out in the usual lines.

As in Trial One population results were measured from the scans of the number of deer present on each line of feed at one minute intervals and continuous sampling measured the time each individual spent eating each diet.

6.4.2 Results - Trial Two

This trial tested further the first question put forward for Trial One, **"can deer learn the nutritional content (protein) of two foods presented simultaneously?"** Another question was also posed having found that the optimum protein intake was 13.6%CP from Trial One, **"do deer take <u>only</u> their optimum protein diet (OP2) when faced with a choice between this and a higher protein diet (HP2)?"**.

6.4.2.1 Fallow deer

Population preference test

The deer at Paultons Park were tested first. It soon became apparent that there was either something wrong with the diets or the deer. On the first day the deer fed for 30 minutes but on Days 2-5 they did not feed at all. Concern for the animals' welfare dictated that the feeding of the test diets could not continue after Day 5, although they had grass and some hay the deer were obviously very hungry. Their normal food was offered which they ate immediately. This indicated that the problem did not lie with the deer but with the test diets.

In order to use the first day's data at least two more days of recordings were needed, so the test diets were fed again after a two week break. The deer fed over two days but for progressively less time (Day 1: maximum 30 minutes, Day 2A [A = second attempt]: maximum 17 minutes, Day 3A: maximum 10 minutes). The preference scores were derived from the total time spent feeding (Table 6.7).

The results were inconclusive, there still appeared to be something wrong with the feed. They were then tested at Stonor.

Table 6.7 Preference scores of fallow deer for OP2 vs HP2 at Paultons Park and Stonor (days 2-4 did not consecutively follow on from Day 1, indicated by the letter A).

Day	Paultons Park	Stonor
1	0.51	0.803
2A	0.405	0.526
3A	0.464	0.686
4A		0.518

On Day 1 at Stonor the deer came to eat, the majority ate for 15 minutes after which time over half moved away. No deer came to feed on Day 2, however over the following 24 hours most of OP2 was eaten but the HP2 was left. As at Paultons, in an attempt to use the data from Day 1, a two day gap was left and the trial was resumed. A small number of deer ate for approximately 15 minutes on Days 2A - 4A (Table 6.7). There was clearly a problem with the diets.

Rowett Research Services had been asked to make up the diets to the required protein levels, both had been made up using fish meal, particularly HP2 (Tables A11.3 - 4, Appendix 11). To the human sense of smell the odour was very strong although the taste was not fishy. It was thought that the fishy odour may have been an aversive stimulus.

The trial was repeated with a different design in an attempt to unravel exactly what was the problem. Using deer at Magdalen only OP2 was fed on Days 1-2, HP2 was fed on Days 3-4, this was to have been followed by the choice test between OP2 and HP2. The hypothesis was that the deer would eat the OP2, as they had at Stonor, and on Day 3 when presented with HP2 would develop an aversion which when presented with a choice would persist. An aversion may also develop for the OP2, if the fishy smell was the stimulus.

On Day 1 at Magdalen (OP2 only) all the deer ate for approximately 30 minutes. On Day 2 OP2 was laid out again but they did not come to eat. They remained in a tight group with the dominant male actively rounding up the group away from the food. This behaviour had also been witnessed at Paultons Park. The HP2 was put down on Day 3 and a small group of mainly young males came to feed for 16 minutes. The rest of the group were kept away by the dominant male. On Day 4, most of the previous day's HP2 remained, more was added to give the equivalent of one day's feed. No deer came to eat it. On Day 5 a dead female was found, *Pasteurella* was suspected and later found to be the case. This is a respiratory disease that deer can carry and can be brought out in times of stress. Due to the presence of this condition the trial was stopped. Although it was unlikely to have been completely due to the stress of the trial it was considered inappropriate to continue. There were two more deaths a few days after the end of the trial.

From the three groups it was concluded that the fishy smell of the diets was an aversive stimulus although the taste was acceptable. In an effort to continue and use the diets for their initial purpose, the foods were flavoured to mask the odour thus encouraging the deer to eat both diets (see Section 6.4.2).

Flavour trials

At Stonor there was a higher preference for OP2 + almond whereas at Paultons there was increased preference for HP2 + almond (Table 6.8). This strongly suggested that the deer were selecting the almond flavouring and not for the content of the different diets.

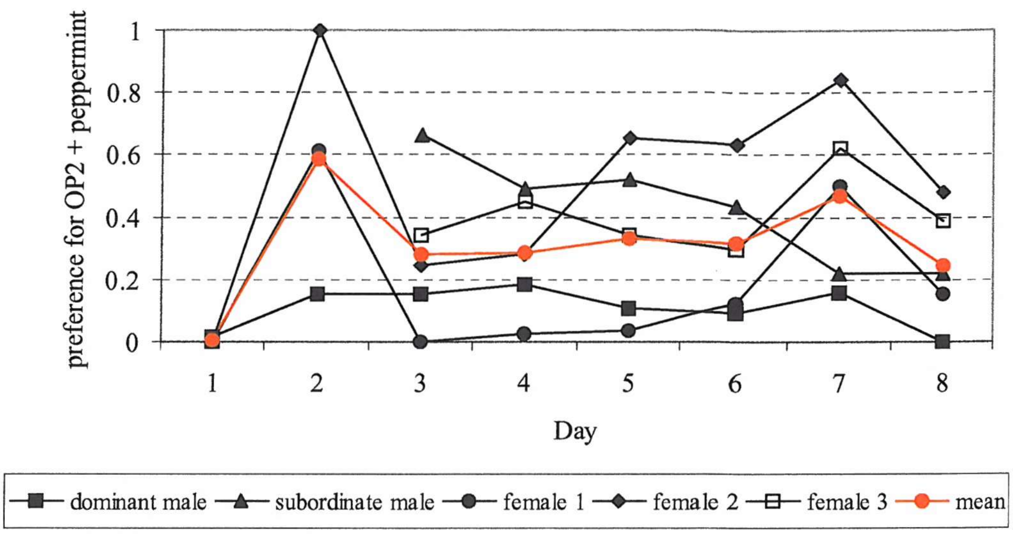
Table 6.8 Preference scores for the deer at Paultons Park and Stonor when the diets were flavoured with almond and peppermint essence

Day	**Paultons Park** **Preference for OP2 + peppermint vs HP2 + almond**	**Stonor** **Preference for OP2 + almond vs HP2 + peppermint**
1	0.05	0.803
2	0.25	none fed
3	0.222	0.634
4	0.333	0.702
5	0.364	none fed
6	0.34	
7	0.4167	
8	0.314	

At Paultons Park the continuous sampling method was used recording actual time spent eating by each individual (Figure 6.9). The subordinate male was prevented from eating for the first two days by the dominant male and on one occasion female 3 fed for less than one minute. All of the females and the dominant male started by eating predominantly or entirely (females) the HP2 + almond. All, except female 2, ate mostly this diet throughout the eight days.

These data were not statistically analysed as the deer at both Paultons Park and Stonor were clearly discriminating using almond flavouring and not the nutrient content (protein) of the diets as put forward in the initial question.

Figure 6.9 Preference scores for OP2 + peppermint as compared to HP2 + almond by the deer at Paultons Park derived from their actual time spent feeding on each diet.



6.4.2.2 Roe deer

In contrast to the fallow deer, the roe at Alice Holt readily ate both OP2 and HP2 without any adverse reaction or need for flavourings. As with Trial One, the Forestry Commission's own video equipment was used. For Trial Two the recordings made were very poor and data on how long each individual spent eating could not be collected. However, the food had been weighed in and out allowing calculation of population preference scores based on the amount of each diet eaten (Table 6.7). The video did show all three deer ate both diets.

After the first day when only OP2 was eaten, the deer settled at a preference score of approximately 0.56 which gave a CP content of 19.47%.

6.4.2.3 Handling trial two

A handling trial was not carried out for the diets used in Trial Two as it was believed no individual deer would eat either diet in adequate amounts to provide sufficient data.

Table 6.9 Preference scores of roe deer for OP2 at Alice Holt and then converted into crude protein (%CP) content eaten.

Day	Preference score	%CP intake
1	1	13.65
2	0.6	18.94
3	0.55	19.6
4	0.63	18.55
5	0.56	19.47
6	0.57	19.34
7	0.56	19.47

6.5 TRIAL THREE

6.5.1 Method - Trial Three

As Trial Two (Section 6.4) was inconclusive Trial Three was essentially a repeat of Trial Two. However, in this case the composition of the diets was changed. The high protein diet, 21.89%CP (HP3) in this case was a commercially available deer feed from Dukes of Botley Agriculture Ltd., Hampshire (Table A11.6, Appendix 11). A corresponding optimum diet, 13.54%CP (OP3) was formulated (Table A11.5, Appendix 11) and manufactured by RRS. OP3 contained as many of the same ingredients as HP3 as possible, but was restricted by the ingredients that RRS possessed and could process through their machines, hence the slight difference in CP content to OP2. As in Trial Two, the high protein diet (HP3) contained more molasses than OP3.

Trial Three also used only choice tests and was conducted on the fallow deer at Paultons Park, Magdalen College, Stonor Park, New Forest Nature Quest and White House Farm. Unfortunately no roe deer were available to take part in this trial. Population preference scores were obtained for all five sites and individual preferences were calculated for each deer at Paultons Park (N=8) and White House Farm (N=3).

The same questions asked in Trial Two were also asked of this trial.

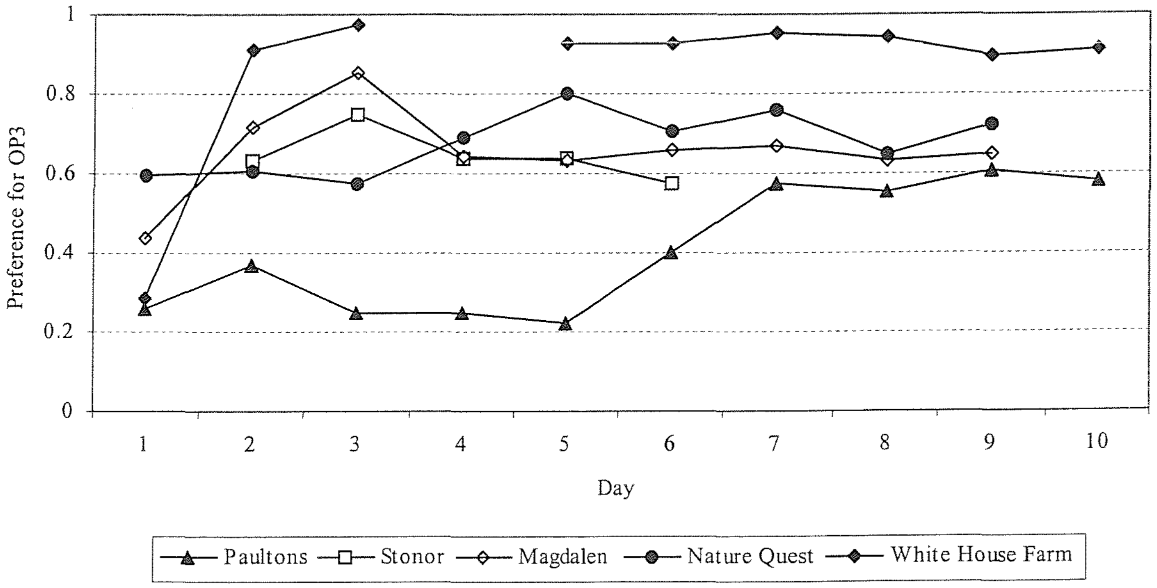
6.5.2 Results - Trial Three

6.5.2.1 Population preferences

As for the previous two trials the population preference scores were taken from scans every minute of the numbers of deer feeding on each line of food. The length of time over which the scans were made was 16 minutes as not only was this the shortest length of time spent feeding (apart from Day 1 at White House Farm) but it was also the most common duration (Table A12.2, Appendix 12). Unfortunately data were not collected for Day 1 at Stonor because the deer did not feed whilst the experimenter and equipment were present and for Day 4 at White House Farm because of problems with the video equipment.

Figure 6.10 shows the population preference scores for OP3 at all five sites. On Day 1 most of the preferences scores for OP3 were below 0.5 indicating that more of the HP3 was eaten than OP3. This was what had been intended by making HP3 sweeter and more palatable. In theory, if the deer were able to discriminate, after taking a diet high in HP3, a switch should occur to eating more OP3.

Figure 6.10 Preference scores for OP3 for the fallow deer at Paultons, Magdalen, Stonor, Nature Quest and White House Farm



Assuming Stonor Day 1 was the same as the majority (*i.e.* <0.5), the deer at Magdalen, Stonor and White House Farm displayed a switch and took mostly OP3 from Day 2 onwards. For Paultons Park there was a switch but much later, between Days 5-7. Although Nature Quest did not show the same pattern as any of the other four sites all the deer did arrive at a similar preference score on Day 9, apart from the deer at White House Farm which showed a very high preference for OP3 (final score = 0.909). The final crude protein intake for Paultons Park was 16.88%; Stonor 17.11%; Magdalen 16.46%; Nature Quest 15.88% and White House Farm 14.38%, the overall mean was 16.1%.

The final preference scores at the three sites were tested for a significant preference for one food over the other (in this case OP3 over HP3). As for Trial One a one sampled t-test was used on the transformed preferences to test the following hypothesis:-

H_0 = no preference was shown for one food over the other

The t-test produced a significant result ($t_4 = 2.834$, $P<0.05$), the null hypothesis was rejected. Therefore, the preference scores for the three sites showed that fallow deer had a preference for OP3 in favour of HP3.

6.5.2.2 Individual preferences from continuous sampling (actual time spent feeding)

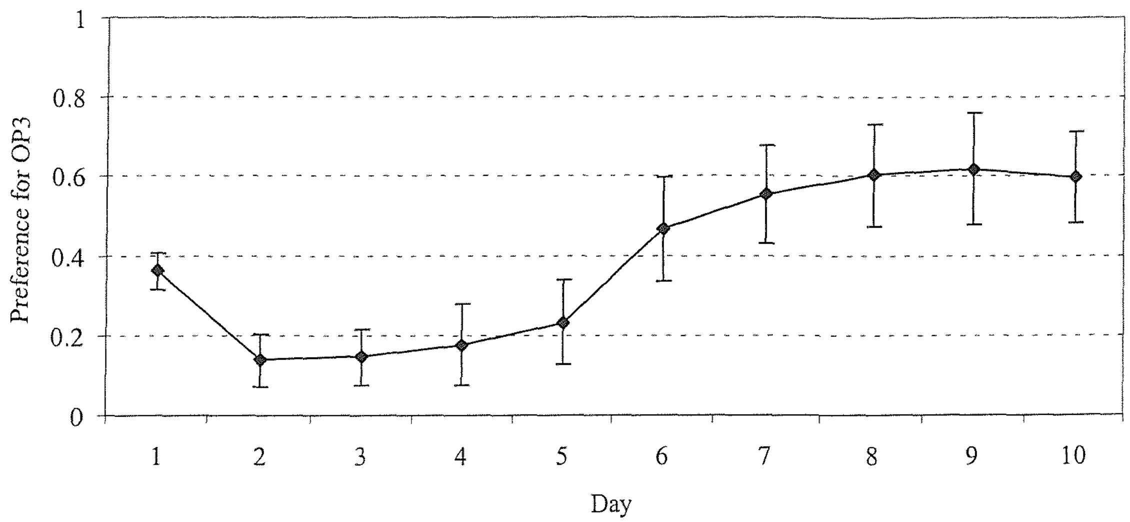
Paultons Park

The mean preference scores from the eight individuals at Paultons Park are shown in Figure 6.11. The standard error bars reveal considerable variation in the scores across individuals, particularly from Day 4 onwards. This variation was investigated using ANOVA on the arcsine square root transformed data and tested the following null hypothesis:-

H_0 = the preference scores for OP3 were not affected by the age or sex of the deer

The deer were divided into age/sex classes: Adult male (N=1), Adult female (N=4) and Fawn (N=3). The ANOVA indicated that there was a significant effect of age/sex ($F_{2,79} = 17.298$, $P<0.001$), thus the null hypothesis was rejected. A Duncan multiple range test revealed that all classes were significantly different from one another. The fawns ate slightly more OP3 than HP3 (mean preference score = 0.59) whereas the male ate predominantly the latter every day (mean preference score = 0.06). The females had a mean preference of 0.32: more HP3 than OP3.

Figure 6.11 Mean preference scores for OP3 (± standard error), using the eight individual deer at Paultons Park.



A second null hypothesis was tested using ANOVA on the transformed data but this addressed the individuals:-

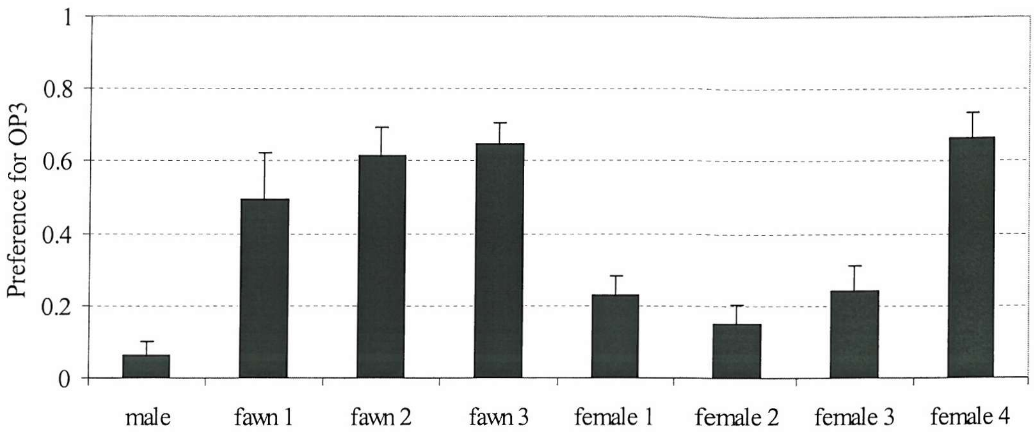
H_0 = there was no difference in the preference scores for OP3 for the eight individuals

As expected from the previous ANOVA the individuals were significantly different ($F_{7,79} = 11.507$, $P<0.001$) so this null hypothesis was also rejected. A post hoc multiple range test showed that all the fawns and female 4 were significantly different to the others by eating higher proportions of OP3. Also the male was significantly different in his preference scores to all except female 2, as mentioned, he took mostly HP3 every day. Figure 6.12 shows clearly the differences in preference between the eight individuals.

Finally, ANOVA looked at the effect of day, whether or not the preference scores changed significantly. The null hypothesis tested was:-

H_0 = there was no significant difference between the days in terms of preference scores of the deer

Figure 6.12 Mean preference scores (± standard error) over 10 days for the individual deer at Paultons Park (N=10).



In this case the null hypothesis was accepted as the ANOVA showed that the preference scores were not significantly different on each day of the trial ($F_{9,79} = 1.828$, NS). This reflects the absence of a reliable switch between OP3 and HP3, certainly until much later than most of the other sites and then not of similar proportions.

As with the population final preference scores those for the individual deer were tested to see if they deviated from no preference (score of 0.50) using a one sample t-test on the transformed data. The same null hypothesis was tested:-

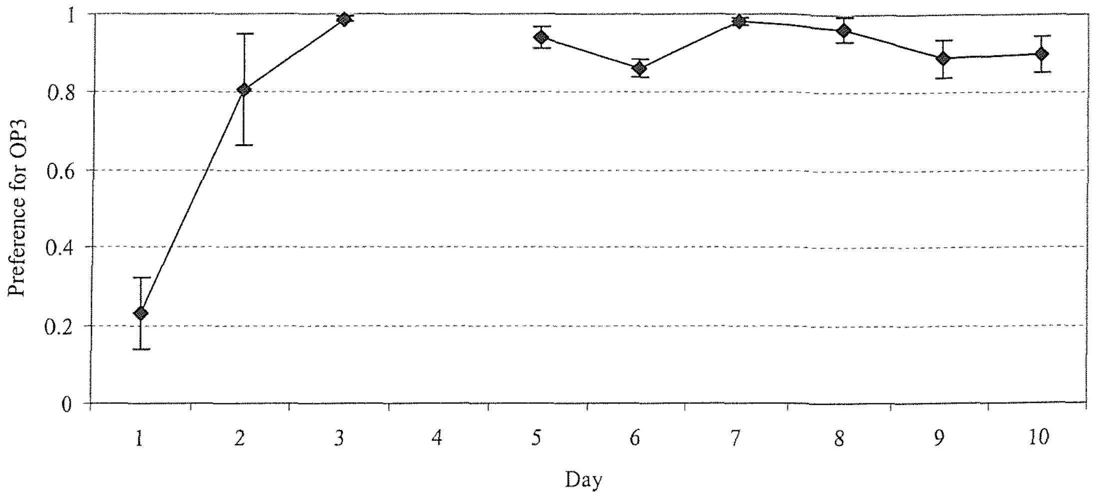
H_0 = no preference was shown for one food over the other

The test produced a non significant result ($t_7 = 0.668$, NS) suggesting there was no preference shown by the group as a whole.

White House Farm

Figure 6.13 displays the mean preference scores for the three fallow deer at this site. The standard error bars show that there was more variation between individuals over the first few days which was the converse of what happened at Paultons Park. The switch from mainly HP3 to OP3 occurred between Days 1 and 2. From Day 3 onwards there was very little variation, all the deer were eating both diets in very similar amounts.

Figure 6.13 Mean preference scores for OP3 (± standard error), using the three individual deer at White House Farm.



As there were no fawns in this group and only three individuals an ANOVA was only carried out to look at the individuals and the effect of day. The same null hypothesis as used for Paultons Park was tested on the transformed data:-

H_0 = there was no difference in the preference scores for OP3 for the three individuals

No significant difference was found between the individuals ($F_{2,26} = 0.222$, NS) so the null hypothesis was accepted, all the deer showed very similar preference scores regardless of gender.

Due to the large difference in preference scores between Days 1 and 2, when the null hypothesis regarding the effect of day was considered there was a significant effect ($F_{2,26} = 9.85$, $P<0.001$), thus the null hypothesis was rejected. The multiple range test separated out Day 1 as having a significantly different preference score to the other nine days.

The final preference scores of the individual deer were tested to see if they deviated from no preference (score of 0.50) using a one sample t-test on the transformed data. The same null hypothesis was tested:-

H_0 = no preference was shown for one food over the other

A significant result ($t_2 = 5.982$, $P<0.05$) was produced for these deer suggesting there was a preference shown by the whole group, OP3 in preference to HP3.

6.5.2.3 Factors affecting preference scores

As for Trial One, a number of factors require consideration that may affect the preference scores for the test diets in Trial Three. The main one was the handling time, this was investigated using method described in Section 6.2.4. The numbers of feeding bouts performed each minute by each individual deer were analysed to consider social hierarchy. The use of each line of feed was also evaluated as if one line was favoured more than the others, this could have influenced the preference scores. Handling trials were performed using the deer at Paultons Park and White House Farm. The data from measuring the individual preference scores for these two groups was used to investigate the social hierarchy and use of lines.

Handling trial three

In contrast to Trial One no one deer ate solely from one dish. The deer ate indiscriminately at Paultons Park and at White House Farm however the amounts eaten could calculated from what remained. Therefore the length of time and number of deer it took to eat each amount (g/deer minutes) was recorded from the video tape. From this a mean rate was calculated in terms of g/min (Table 6.10).

Table 6.10 Rates of consumption of the test diets in Handling trial three (OP3 and HP3).

		Rate (g/min)	
Day	**Diet**	**Paultons Park**	**White House Farm**
1	OP3	79.92	45.17
2	HP3	78.77	36.13
3	OP3	74.93	35.71
4	HP3	70.34	64.5

A Levene test indicated that the variances were homogeneous so the data were analysed using an independent t-test. The following null hypothesis was tested:-

H_0 = the handling time for both diets was the same

The t-test found no significant difference between the handling rates of OP3 and HP3 ($t_6 = 0.245$, NS), therefore the null hypothesis was accepted. A further test was done to assess differences between the two groups of deer (Paultons Park and White House Farm). Again a Levene test showed that the variances were not significantly different and so an independent t-test was performed. The null hypothesis being tested was:-

H_0 = the handling times for the deer at Paultons Park and White House Farm were the same.

This test indicated that there was a significant difference between the two groups ($t_6 = -4.326$, $P<0.005$), therefore the null hypothesis was rejected. Table 6.10 shows that the deer at White House Farm ate both diets much more quickly than those at Paultons Park.

Feeding bouts

The numbers of feeding bouts per minute were explored at Paultons Park and White House Farm. The data were kept separate as they would provide information on social hierarchy which can only be investigated within a group.

Paultons Park

The number of feeding bouts per minute were measured for all eight deer and those of the male are highlighted by the red line in Figure 6.14.

These data were explored for differences between the individuals and over time using ANOVA on $\log_{10}$ transformed data.

H_0 = there were no differences in the numer of feeding bouts performed per minute by the eight individuals

The ANOVA showed that there was a significant difference between the individuals in terms of the numbers of feeding bouts they performed per minute ($F_{7,79} = 3.274$, $P<0.005$) and so the null hypothesis was rejected. A multiple range test (Table 6.11) revealed that the smallest fawn (fawn 1)

Figure 6.14 Number of feeding bouts per minute for each of the deer at Paultons Park, with the line of the male highlighted.

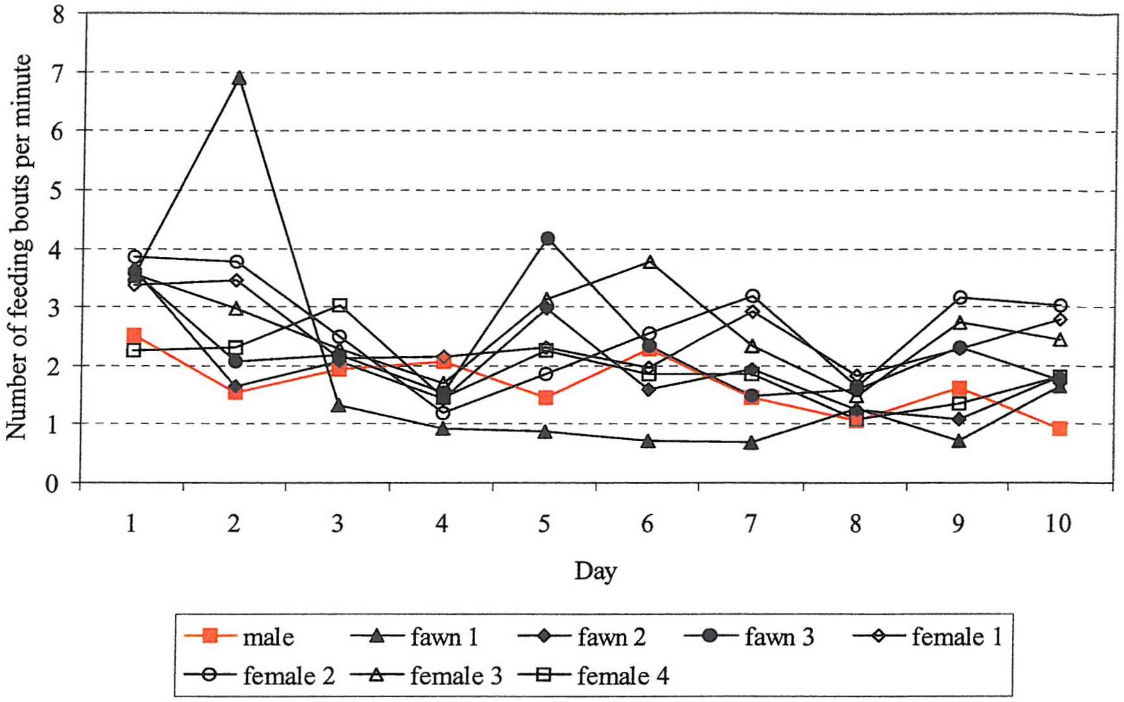


Table 6.11 Multiple range (Duncan) test for the effect of **individual** showing the back transformed mean feeding bout length (means followed by the same letter are not significantly different from each other).

Deer	Mean value
Female 3	2.546 a
Female 2	2.521 a
Female 1	2.467 a
Fawn 3	2.174 a b
Female 4	1.853 a b c
Fawn 2	1.826 a b c
Male	1.6.16 b c
Fawn 1	1.350 c

performed the fewest bouts and was significantly different to one other fawn (fawn 3) and three of the females. This was despite its unsettled and comparatively uncharacteristic behaviour on Day 2 (Figure 6.14).

ANOVA also tested the following null hypothesis:-

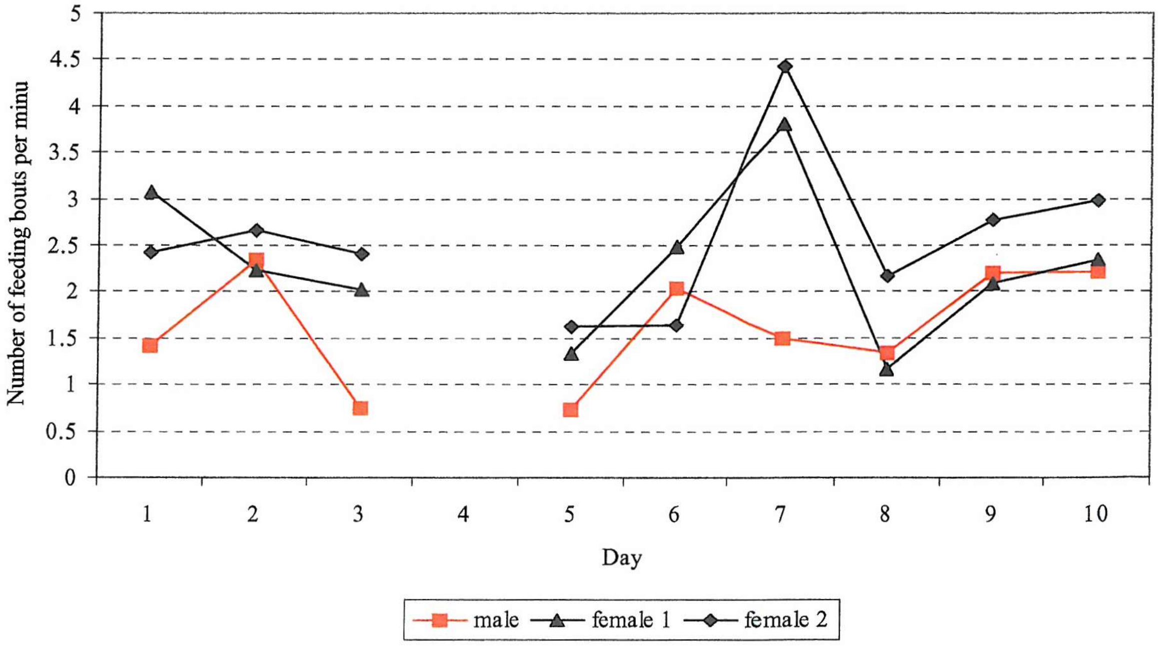
H_0 = the deer performed the same number of feeding bouts per minute each day

There was a significant effect of day ($F_{9,79} = 3.42$, $P<0.005$) and so the null hypothesis was rejected. A multiple range test showed that Days 4 and 8 had the fewest of feeding bouts overall (means: Day 4 = 1.56; Day 8 = 1.41) and were significantly different to Day 1 which produced the highest number (mean = 3.3).

White House Farm

Figure 6.15 displays the feeding bouts per minute for the three deer at this site. It clearly shows that the two females were very similar in the numbers they performed, they closely followed each other. Overall the male performed the least bouts.

Figure 6.15 Number of feeding bouts per minute for each deer at White House Farm, with the line of the male highlighted



The data were tested using ANOVA on $\log_{10}$ transformed data as for Paultons Park and addressed the same null hypotheses. The individual deer did perform significantly different numbers of feeding bouts per minute ($F_{2,26} = 4.188$, $P<0.05$), so that null hypothesis was rejected. The multiple range test indicated that the male performed the least (mean = 1.49) as observed in Figure 6.18. The females were not significantly different from each other (means: female 1 = 2.15; female 2 = 2.46).

There was no significant effect of day ($F_{2,26} = 4.188$, $P<0.05$), thus the null hypothesis was accepted, the deer performed similar numbers of feeding bouts per minute each day, Day 5 (mean 1.16) showed the least and Day 7 the most (mean 2.93) but were not significantly different.

Use of lines

As for Trial One the duration of time the deer spent on each line of feed was considered, in this case it was investigated at Paultons Park and White House Farm. The following null hypothesis was tested for both groups of deer:-

H_0 = all lines of feed were used to the same degree over the trial

This was investigated at Paultons Park using a Kruskal Wallis test and a significant result was produced ($\chi^2_3 = 5.328$, NS) thereby accepting the null hypothesis. For the deer at White House Farm a Mann Whitney test was performed as there were just two lines. This produced a non significant result ($U_1 = 31$, NS), the null hypothesis was accepted: both lines were used similarly.

6.6 DISCUSSION

This section considers fallow and roe deer separately in terms of each the feeding trials. Fallow deer were involved in all three trials (Section 6.6.1), whereas roe were only used in the first two (Section 6.6.2). Section 6.6.3 considers the factors that may have affected the results, this discusses handling trials, social hierarchies and use of the lines.

6.6.1 Fallow deer

6.6.1.1 Trial One

The theory that the deer would initially take more low protein diet because it was made more attractive (Section 6.1), was bourne out by the deer at Magdalen and Stonor, the switch from low to high protein occurred very quickly, in both cases after the first day (Figure 6.4). However, for the deer at Paultons the result was very different, using both types of measurement, scans and continuous sampling, the results were the same: they started by selecting nearly all the high protein diet then after the third day took in more of the low protein until a balance was achieved (Day 10 will be discussed later).

From the data for all three fallow parks it can be concluded that the deer were able to discriminate between the two diets but whether this was because of the different protein levels is difficult to say. It is not possible to categorically state that the deer at Paultons and Magdalen learned the difference in protein content of the two foods when presented simultaneously or that those at Stonor learned the protein content of the two foods when presented one at a time. However, the results do strongly suggest some degree of learning as indicated by the switches in and stabilising out of the preferences. The final preferences were all very similar and significantly different from a score of 0.5 (achieved through random feeding), highly suggestive of underlying mechanisms operating. One of the problems with preference tests is that there is a 'ceiling effect', that is there are rigid minimum and maximum values, in this case zero and one. The results obtained here may have been quite different if the measurements could have been on a continuous scale, *e.g. ad libitum* feed available and volume recorded. However, the use of preference scores was believed to be the best way of representing the data obtained here.

The reason why the daily preference scores were different for the three parks is unclear. The only obvious difference in conditions was that the deer at Paultons were fewer in number and their enclosure was relatively much smaller. These deer may have been exhibiting neophobia (fear of novel food) at the beginning of the trial and this may have been influenced by their normal background diet that contained similar sized pellets but also flaked maize and bran, whereas the other park deer had pellets alone. This study suggests that social learning may be taking place by the deer at Paultons as many appear to be following the dominant male (Figure 6.8), especially the subordinate male, but this could be for other reasons (*e.g.* ongoing dominance relationship).

At Paultons all the deer exhibited similar preferences for the high protein especially for the first three days, this similarity in behaviour could suggest neophobia (Figure 6.5). But on Day 10, when there was much disturbance outside the enclosure, the deer reverted to the same mean preference as in the first instance. This suggests that the reason for the preference initially may have been that the deer were wary and nervous because of the new feed (neophobia) and feeding arrangements, despite attempts to keep the procedures as usual as possible, and on Day 10 because of outside disturbances making them nervous.

The data for the differences in preference between males, females and juveniles at Paultons suggest that the males require more protein than the females, but that the juveniles require more than the males. Feeding trials with white-tailed deer fawns has shown that males preferred a higher protein diet than females (Ullrey *et al*, 1967). This was also shown by Verme and Ullrey (1972), males producing maximum gain in body weight with high protein diets and females with lower protein. These data were admittedly for fawns but Orskov (1982) states that for all domestic ruminants, females contain more fat and less protein as opposed to males so it may be that the differences in body size determine the protein requirement. For females protein requirement differs with different stages of their lives, such as when they are lactating; at this time females require higher amounts of protein (17% crude protein: Adam, 1988, see Table 5.3). At the time of this feeding trial it was assumed that most (if not all) of the females would have been pregnant. However only by repeating the trials at other times of the year could this winter data have shown if they required high levels of protein at this time. Increase in requirement only becomes apparent in the latter part of pregnancy (Adam, 1988); the females still had another 3-4 months gestation. The protein requirement of females at this time is 6-10%CP (Adam, 1988; Reinken *et al*, 1990), all the females ended up taking a combination of diets that were much higher than these values. As for the difference between the adults and the young, the latter are of course still growing and would require higher levels of protein. The reason that the young female had the highest intake of protein may have been a combination of the facts that she was possibly pregnant but was also still very young and therefore still growing.

6.6.1.2 Trial Two

Unfortunately Trial Two was unsuccessful in showing what was intended, *i.e.* a complete shift to the optimum protein level arrived at in Trial One. The reason for this was the aversive nature of the diets. Flavouring the foods managed to mask the fishy smell that was thought to be the aversive stimulus but the preference for almond flavour outweighed the preference for different levels of protein. The only conclusion from this trial for the fallow deer was that they prefer almond to peppermint flavouring and this should not be used in feeding trials where flavouring is needed. A trial carried out by Arnold *et al* (1980) looking at different flavours, found that sheep preferred flavours other than peppermint.

The literature suggests that the fishy smell was not the aversive stimulus as to produce a strong aversion to the odour there must be immediate aversive post-ingestive feedback, *e.g.* vomiting (Provenza *et al*, 1992; Provenza, 1995). As this was not witnessed the stimulus was more likely taste. Learning with long delays is characteristic of the taste-feedback system as digestion and absorption are relatively slow processes (Garcia, 1989 in Provenza, 1995), conditioned aversion can occur even if post-ingestive feedback is delayed for up to 12 hours (Rozin, 1976; Zahorik and Houpt, 1981; Burritt and Provenza, 1991). In the current study the diets were eaten on Day 1 at all sites, but not subsequently, suggesting a strong aversion to the taste of the foods. It is highly likely that this will have become associated with the fishy odour and only when the odour was masked through flavouring did the deer eat the food. The reason why the deer did then eat the diets for several days (although for shorter durations than the first day) is unclear. A number of reasons are suggested:-

a) only a small number of animals at each site experienced the initial aversive post-ingestive feedback that stopped them eating, and the remainder of the group copied this behaviour. This may have been more probable if the animals that were sick were higher ranking individuals. On second exposure some deer ate the food and the averted animals then sampled the food, after experiencing no adverse reaction they continued, thus terminating the aversion (Ralphs and Provenza, 1999).

b) there was a trade-off between the pleasant experiences the flavours provided (particularly almond) and the negative experiences from the smell/taste. This may also explain why over time the deer ate the diets less and less, the trade-off was becoming unbalanced.

6.6.1.3 Trial Three

As with Trial One the theory regrading switching from one diet to the other occurred in Trial Three at Magdalen, White House Farm, Paultons and probably Stonor. In most cases this was after the first day, however at Paultons it was much later. This site also produced different initial results to the other sites in Trial One, suggesting that a difference in the makeup of the group or habitat was a contributing factor. The only site that approximated what was expected was White House Farm. Here the deer almost exclusively took the optimum protein diet which was suggested from Trial One. This was also the case when the individuals were investigated separately. The other sites arrived at a final protein intake between 15.88 - 17.11%CP. These levels came from preference scores of 0.58 - 0.72. The final scores in the first trial were 0.64 - 0.69. It is possible that when deer are given a choice of two diets they always take similar proportions (*e.g.* 60:40) irrespective of what the diets contain, hence the similar preference scores in both trials. However, as White House Farm produced very different scores to the others it is more likely that the results obtained for the other sites are true for those deer and the similarity was a coincidence.

Trial Three supports Trial One in showing that the deer were able to discriminate between two diets. In Trial One it was not possible to establish if this was due to the different protein contents of the diets, however the results from Trial Three suggest that this may be more conceivable as here the deer at each site also expressed very similar preference scores. Although the diets were designed to vary only in their protein concentrations simply by doing so the amounts of the ingredients were different and these may have accounted for the discrimination between the foods, this would be impossible to unravel without complex feeding trials.

The reason why White House Farm was so different is not obvious, there were only three individuals and there was no evidence of any social displacement. Therefore these deer may have been able to feed much more freely than those at any of the other sites. The individual data at Paultons suggest that some of the deer fed in a similar way to those at White House Farm; two fawns and female 4 took diets that were much closer to the optimum than the rest of the group. For Trial One it was suggested that many deer followed the dominant male, this also appeared to be the case for Trial Three, where three of the females fed in the same manner as he did. Only the apparent subordinates (fawns and female 4) fed apparently how they wanted, assuming that this was what the similarity of their preference scores with White House Farm indicated (See Section 6.5.2.2). The male took almost exclusively the highest protein diet possible and did not show a switch although it is not possible to say he did not learn. The reason for this is not evident. At the time of year the trial was carried out (February), the protein requirement of males is 6-11%CP (Adam, 1988; Reinken *et al*, 1990), much lower than that taken. This diet was designed to be more pleasant tasting (sweeter) so the male may simply have been exploiting this hedonisitc quality. Hedonic value of food is very difficult to measure, but Baumont (1996) states that sensory properties of feed can stimulate or depress hedonic behaviour. He goes on to say the role of hedonic behaviour on intake may be of more importance in choice situation, as in the current study.

The data for the individuals at Paultons was much more variable than it was in Trial One. The group of deer consisted of some different individuals in both trials. The group for Trial One were more stable and had been together for longer than the group in Trial Three. The male in Trial Three was already established when the four females were introduced the previous year, therefore they may have been more likely to take his lead, particularly with the introduction of unusual feed and feeding regimen. However this does not account for the behaviour of the subordinates (see Section 6.5.2.2).

Trial One found that the males took a higher protein diet than the females, which was also the case in Trial Three, but that the fawns took an even higher diet which clearly was not found in Trial Three. It is assumed that the females would have been mid way through pregnancy and so would have required 6-10%CP (Adam, 1988; Reinken *et al*, 1990). The mean intake was two times their requirement (female 1, 19.99%CP; female 2, 20.63%CP; female 3, 19.88%CP) these amounts are not even required at peak times during lactation so the females were clearly not taking these diets through need.

6.6.2 Roe deer

6.6.2.1 Trial One

The responses of the roe deer were not as clear as those of the fallow deer. Here one of the females (collared female) showed no signs of discrimination and learning. The reasons for this may be due to an underlying social hierarchy. The other female and the younger male (her son) showed similar preferences to each other towards the end of the trial. They both showed an almost exclusive preference for the high protein diet, 15.71%CP, a much higher level than that preferred by the fallow deer (13.6%CP). These deer are very different types of feeders as discussed in Section 1.2.2 and their requirement for a high protein diet may be a result of this, although further studies would be necessary to test this assumption.

The fact that the individual roe deer would not eat the two diets presented to them was very disappointing but this too may indicate how very different they are to fallow in terms of their feeding behaviour. It also suggests strongly that without the influence of conspecifics around them, they exhibit neophobia.

6.6.2.2 Trial Two

In complete contrast to the fallow deer, roe deer ate the two diets in Trial Two without aversion to either. The reason for this is unclear. The roe deer had adequate natural forage in their local environment to sustain them if necessary. It suggests that in some way the sensory capabilities of the roe were different to that of the fallow, either they were not as sensitive to the odour or they had a wider tolerance. Roe deer are known to have a wider tolerance of tannins than other deer (Duncan *et al*, 1998), perhaps this extends to other chemical compounds.

The roe deer finally took a diet that was very high in protein (19.47%CP). The results from Trial One showed that two of these deer (the male and his mother) took predominantly a diet as high in protein as was possible. The suggestion made in Section 6.6.2.1 that roe deer require higher protein than fallow appears to have been reinforced by the data from Trial Two. Small ruminants are considered to select diet of higher quality than larger ruminants (Provenza and Balph, 1987).

6.6.3 Considerations of protocol and factors that may affect the results

Due to the nature of the field study the preference tests could not be carried out using the more conventional method as used for rats (*i.e.* feeding could not be stopped after half the food was eaten nor could the food generally be recovered to calculate intake). Both diets were left out on the ground (fallow deer) and all had been eaten by the time the next test was due to start the following morning. This suggests that perhaps no learning took place; a deer could have fulfilled its daily protein requirement later that day by finishing off all the food. Therefore at the end of each day it was possible that each deer was not deprived of protein nor had they had an excess; they had compensated and obtained their usual requirement. It is possible that at the end of the day when digestion and absorption had occurred, the deer had learned very little about the two test diets. However, the different preference scores over the days in Trial One and Three, plus the switch from taking one diet to the other by most of the deer, suggests that learning had occurred. Whether this was due to the different protein levels is another matter.

Another factor that may have affected the preference scores was the social hierarchy within each group. The deer at Paultons and White House Farm were considered in regard to this, using the number of bouts performed per minute as a measure.

A dominant individual has priority of access to a resource (Abercrombie *et al*, 1983) and in this case the resource was food. Hierarchies in deer have been studied particularly in red deer. A study by Thouless (1990) with red deer females showed that the subordinates were vigilant even with their heads down feeding, and that they were conscious of the dominant individual's position, moving when she got closer. In the current study the slightest movement by the dominant male often resulted in the other individuals moving along or off a line of feed and so ending a feeding bout. Thouless (1990) also stated that red deer females were more likely to move away and stop feeding if their nearest neighbour was socially dominant. This was also observed in all the trials at Paultons and White House Farm, for example, one of the subordinate individuals would move away from the dominant male and would encounter several individuals more dominant than itself, thus creating more (but shorter) feeding bouts per minute. This behaviour was suggested at Paultons in Trial One and Trial Three and also at White House Farm where the dominant male in general performed the lowest or second lowest number of bouts per minute; the subordinate male at Paultons in Trial One closely followed the pattern set out by the dominant individual; and a subordinate such as fawn 2, Trial One) performed a consistently high number of feeding bouts in comparison to the rest of the group. In Trial One, female 1 showed the highest mean number of bouts per minute being consistently high throughout the trial. If a high number of bouts per minute was indicative of increased vigilance then it may be possible that this female was the dominant one and as such one her roles was to be aware of the group's surroundings. At other times of the year this role would have fallen to the dominant male (*e.g.* during the rut) but at the time of this trial the rut had occurred and the group's hierarchy should have been stable. So, at this time the dominant male could eat without interruptions from either the rival male or the need to be vigilant, hence his consistently low number of bouts per minute after Day 1 and even on Day 10 when there was some disturbance around the enclosure.

Female 4 at Paultons in Trial Three has already been singled out as being different to the other three females in the group. Her preference scores were similar to those of the fawns and did not approximate those of the other three females. Moreover, she was always the last to arrive on the lines of feed. These two findings suggested that she was a subordinate individual. In contrast to female 1 from Trial One (who was regarded as dominant) she performed fewer feeding bouts. This adds credence to the earlier suggestion that dominant individuals have to be more vigilant and therefore perform more short feeding bouts. In addition, it has already been mentioned in Section 6.6.1.3 that female 4 (and the fawns) took a diet that resembled that of the deer at White House Farm, whereas the other females appeared to follow the dominant male's choice of diet. If it is assumed that the correct choice was to take more of the optimum diet and that the male had his own agenda, then the subordinates were actually taking the better quality diet. In most social groups the dominant individuals acquire better quality resources than their subordinates (Appleby, 1980; Thouless, 1990). However, in this case, if the optimum diet was deemed to be better than what was an excessively high protein diet then the subordinates have been given free access to it. This apparent anomaly has been described for pronghorns (*Antilocapra americana*) by Dennehy (2001). Although high ranking individuals acquired high quality diets he found that the low ranked females obtained better quality diets than their middle ranking conspecifics. He suggested that this was because they spent less time contesting dominance or being vigilant and consequently had more time to forage. It is believed that this was the case here, particularly for Trial Three, the three females were closely following the male, perhaps their internal rankings were in a state of flux, therefore the subordinates were left to do as they pleased and they ate the expected diet. Ideally this hypothesis should be tested on the other groups but unfortunately they were much larger groups and it was impossible to follow each individual every day and define a social hierarchy for the group.

Appleby (1980) stated that the frequency of displacements (dominant individuals displacing subordinate ones) differs with food availability and quality. It is believed that sufficient food was provided in each feeding trial to eliminate, or at least reduce, this cause for displacements. Whether or not the quality had any effect cannot be determined.

Although the social hierarchy was not thoroughly investigated it was nevertheless considered to be a very important aspect of the feeding trials. The population preference scores were regarded as being a true representation of the overall preference for the test diets, but on an individual level the preference scores obtained may very easily have been affected by social constraints.

Other factors that were considered to have possible effects on the preference exhibited by the deer were the handling time of each diet and also the use of the lines of feed. The handling trials carried out on the deer at Paultons and White House Farm showed that there were no differences in the handling times of the two test diets in Trials One and Three. The use of lines of feed investigated at Paultons (Trials One and Three) and White House Farm (Trial Three) was not believed to have had any effect on the preference scores. Therefore other than the possibility of social hierarchy influencing the scores, there are no other factors affecting the results from Trials One or Three for the fallow deer and the methodology devised was well suited to testing the hypotheses about diet selection.

6.6.4 Summary and general discussion

The overall aim of this chapter was to answer the following question **"can fallow and roe deer select specific foods based upon the nutrient content?"** The data for fallow and, to some extent, roe deer clearly showed that these deer can discriminate between foods of different nutritional content when presented either one at a time or simultaneously. The trials were designed using diets that varied only in protein. However the results are not conclusive that preferences were driven solely by this nutrient. Another question was asked to try and unravel this further, **"do fallow and roe deer take only their optimum protein diet when faced with a choice between this and a much higher protein diet?"** Only one group of deer (White House Farm, N=3) showed an almost complete switch to the optimum protein diet, the other populations showed similar final preference scores. This still does not positively identify protein as the selection factor, as it only occurred in one population. However the results obtained for this question do reinforce the earlier findings that fallow and roe deer certainly can discriminate between two different foods. These species of deer clearly have the ability to select different foods. As almost every population studied arrived at a similar final preference score, this study tentatively states that protein concentration can be a basis for diet selection.

Discriminatory ability of deer has been confirmed since the current feeding trials took place, by Berteaux *et al* (1998) working on white-tailed deer with a choice of four diets. These diets varied in energy and protein, the deer selected combinations of the diets that were higher in energy but lower in protein, attributing energy as the limiting factor at the time of the trial. The paired diets in the current study were as similar in energy content as possible.

The current study also strongly suggested that these deer learnt about the content of each pair of foods presented one at a time and simultaneously. The evidence for this is based on the switch from more of one diet to the other which was witnessed in most of the deer populations and was the intention behind the design of the trials. It is proposed that the deer learnt through post-ingestive feedback mechanisms (Provenza *et al*, 1994; Provenza 1995) that generally occurred after the first day of eating the diets. This was very rapid, learning by six days has been demonstrated using feeding trials with sheep (Edwards *et al*, 1997). Learning is very complex but certainly has its place in diet selection and will be discussed in more detail in Chapter 7.

Both species of deer took two to three times the published crude protein requirement for the time of year (6-11%CP: Adam, 1980; Reinken *et al*, 1990), with roe deer choosing a higher protein diet (16-19%CP) over fallow (14-16%CP). The reason why both species selected higher protein diets than necessary could simply have been due to availability.

CHAPTER 7 GENERAL DISCUSSION

This general discussion firstly addresses the main findings from each chapter (Section 7.1). The following section specifically discusses diet selection in this current study and the applicability of the five models described in Chapter 1 (Section 7.2). Possible applications of the findings are described in Section 7.3 and finally a description of possible future work is provided in Section 7.4.

7.1 MAIN FINDINGS FROM EACH CHAPTER

7.1.1 Diet composition

The general conclusion from Chapter 2 was that the faecal analysis technique was the right one for this particular study, moreover it was shown to be a reliable method when the results were compared to the findings of other researchers. In all the analyses the diets of fallow deer were different to those of roe deer. Differences also arose between the fallow sites (Brinken and Denny Lodge), but the roe sites (Great Covert and Squabb) were generally much more similar to each other. The fallow sites, despite both being in the New Forest were very different in their structure whereas the roe sites were more uniform. The results suggested that availability of plants played more of a key role in diet composition in this study than the fact that fallow and roe deer are different kinds of feeders (roe: concentrate selector; fallow: intermediate; see Section 7.2.3). The numbers of plant species in the diet and the proportions in which each were eaten varied seasonally. In spring, compared to winter, a higher number of plants were found and generally greater amounts of many were eaten.

The most notable departures from published accounts of fallow and roe deer diets were the absence of grasses for the fallow deer, particularly at Brinken, and the high amount of conifers at one of the roe sites, Great Covert. It was believed that these aberrations were a result of the hurricane in 1990. This storm brought down many trees most of which were not uprooted sufficiently to kill them so carried on growing, becoming more accessible to deer. This was witnessed at Brinken with Holly and at Great Covert with Western Hemlock and Douglas fir, these plants formed major parts of the diets for both populations of deer. The diet composition of roe deer at Squabb provided more evidence for this theory as here very few trees had fallen either completely or partly, and as a result the main component was Bramble, in agreement with the deer studied by Hosey (1974, 1981).

7.1.2 Availability of vegetation

The main conclusion from Chapter 3 was that diet selection at both fallow deer sites and at Squabb (roe deer) was not based solely upon the availability of vegetation. Only at Great Covert (roe deer) was there any suggestion of a link between numbers of available species and their consumption and this was only during winter. In the summer it was very unlikely that the diet was selected on the basis of availability as during this season there were a multitude of plant species available and smaller numbers were actually eaten.

7.1.3 Chemical analysis of vegetation

Seasonal variation was found in all the nutrients measured. This variation was greatest in plants that had distinct annual growth cycles, such as deciduous trees, and least in conifers, which showed more consistency over the year. All of the nutrients, except calcium, peaked in the spring with new growth and decreased with senescence as the year progressed. The opposite was true for calcium which increased as plants matured. Comparison with the literature indicated that the techniques used were reliable and that the results were valid. However, there was some speculation about the concentrations of phosphorus which were later considered to be too small to be reliable. Brinken produced plants that were relatively nutrient poor, the reason for this was thought to be the acidic soil.

7.1.4 Diet selection in the natural environment

Combining the data on diet composition (Chapter 2) with the nutrient content of each important species eaten (Chapter 4) and considering availability (Chapter 3), demonstrated that fallow and roe deer selected different diets at different times of the year, particularly in winter, based at least partly on the nutrient content of the plants. It also highlighted the fact that diet selection is multifaceted and that despite earlier speculation, availability also played a key role, particularly in winter. The most commonly eaten foods were those most available in winter and all had the necessary nutrient levels (of those measured) for maintenance of both fallow and roe deer. It can be argued that this may be a coincidence and not by design, however the feeding trials showed that both species of deer possessed the ability to select diets that jointly contained a specific level of protein. Therefore, the deer may have had little choice when it came to the winter due to availability, but of what was available, they took the necessary levels of nutrients for maintenance.

Many authors have noted that protein (Hellmers, 1940; Torgersen and Pfander, 1971; Parfitt, in Putman, 1986a) and other nutrients (phosphorus: Torgersen and Pfander, 1971) are limited in the winter and that deer can experience deficiencies at this time. The current study found that protein was not limiting in the winter, there were adequate supplies from the three main components of the diet alone, thus all the deer could take a diet that was not deficient. In fact, the opposite of most findings was true here. Protein levels at the most critical times in the life cycle of the female deer, were found to be below the requirements for late pregnancy and lactation. This was particularly the case for roe deer if the assumption that this species required higher levels than fallow deer, is correct.

Deficiencies were also apparent for calcium at the time of lactation for fallow and roe deer and antler growth for fallow deer. It is assumed that requirements were met from stored nutrients. Stephenson and Brown (1984) have shown that white-tailed deer possess physiological mechanisms for conserving and mobilising calcium from bone to antlers and other sites in times of need. Interestingly, unlike protein at these times, although there was sufficient calcium in the three most commonly eaten plants the majority of the deer chose diets that were deficient. To acquire the correct levels of calcium may have demanded that they ate plants in such amounts that could have been aversive in some way, *e.g.* unpalatable, high in toxins. This choice of an apparently deficient diet also occurred with magnesium, the deer at all of the sites except Squabb took deficient diets but not at such critical times as calcium.

7.1.5 Diet selection using feeding trials

In all the trials the deer exceeded their protein requirements for the time of year, in some cases they were taking twice that needed. In Trial One young fallow deer took higher protein diets than adult males who in turn took higher concentrations than adult females. This was not found in Trial Three, the younger animals took what was expected (*i.e.* more optimum protein diet) and the older male and females took a much higher protein diet. Although only one population of roe deer could be tested, the two trials clearly indicate that roe deer have higher protein requirements than fallow deer (see

Section 7.2.3). The outcome from Trial Two was not expected, however it showed some interesting behaviour and gave an insight into conditioned food aversion, it also allowed flavours to be used to further investigate this phenomenon. This will be discussed in Section 7.2.4 in more detail.

7.2 APPLICATION OF MODELS OF DIET SELECTION

Diet Selection in the natural environment and that explored experimentally through feeding trials will now be discussed in terms of the models of diet selection put forward in Chapter 1 (Introduction): hedyphagia (section 7.2.1), euphagia (Section 7.2.2), body morphophysiology (Section 7.2.3), learning (Section 7.2.4) and optimal foraging (Section 7.2.5). No one model was tested specifically, all will be considered separately and brought together in a summary conclusion at the end.

7.2.1 Euphagia

Specific hungers generated within the body (euphagia) are unlikely to have been operating in the diet selection of the deer in this study, either in the feeding trials or in their natural environment. Euphagia does not involve associative learning between taste, smell and post-ingestive consequences, it is innate and assumes the animals have no memory of what they eat. The diet composition of the deer feeding in their natural environments included most plants all year round in varying amounts. If euphagia were operating solely it would assume the deer had no recall of what was eaten each day, and specific hungers operated daily dictating what was eaten. This appears to be very unproductive, however it would be more beneficial if it operated for particular requirements (*e.g.* nutrients) at certain times, *i.e.* switched on when the need arose such as in times of deficiency. Animals, including ruminants, have only been shown to display specific hunger for sodium (sheep: Denton and Sabine, 1963; cattle: Bell, 1984). From this study it was not possible to completely rule out specific hungers operating in the natural environment at certain times of year or of an animal's lifecycle, however the literature suggests that it was highly improbable.

In the feeding trials it is believed that fallow and roe deer were not showing specific hunger for protein as they changed their preference throughout the trial, usually after the first day. Although it is possible that the taste, smell or post-ingestive feedback of the diets were not registered and remembered, it is highly implausible.

7.2.2 Hedyphagia

Another simplistic approach to diet selection is hedyphagia, where there are immediate sensory consequences when an animal puts food in its mouth, *e.g.* it is 'pleasing' to the olfactory, gustatory and tactile senses. Researchers have hypothesised that with this model through natural selection plants that are nutritious would taste good and those that contain toxins would taste bad, however with this there is a suggestion of learning through association. It is not possible to say whether or not hedyphagia was being practised by fallow and roe deer feeding naturally, the plants eaten were not analysed for their taste (sweet or bitter) or their smell nor could the senses of deer have been simulated in respect of these measures, only the human opinion could be concluded. Hedyphagia also considers tactile sensory consequences, *e.g.* thorns, stinging hairs. From the diet composition data there were certain plants that were available that were not eaten, *e.g.* nettles (*Urtica dioica*), noted for their stinging hairs, but although they did not appear in the diet of either species of deer this is not to say that these physical properties were the reason why. Fallow deer at Brinken in particular, ate a lot of Holly which has spines, roe deer ate significant amounts of Bramble, which also has spines. Hedyphagia cannot be dismissed in the interpretation of the diet selection by fallow and roe deer in their natural environment as this study did not provide sufficient evidence for its presence or absence, however it would be extremely difficult to demonstrate.

The selection of diets in the feeding trials appeared to have shown hedyphagia operating, at least initially. One of the diets in each pair was made sweeter, albeit to human sensory abilities, to force the deer to take this diet first. This occurred at most of the sites in each trial, suggesting that they immediately found the taste pleasing. For the male at Paultons Park in Trial Three, this hedonistic reward was hypothesised as the reason why he took this diet throughout without making the more usual switch to the expected diet. Hedyphagia assumes there are no interactions between the odour, taste and consequences of eating, this was clearly evident for this male deer in this trial. However in the absence of any other examples the presence of hedyphagia was inconclusive

7.2.3 Body morphophysiology and size

The evidence for or against this model come from interspecies comparisons. Fallow and roe deer vary in size, fallow being the larger and are classified as different kinds of feeders (Hofmann, 1985, 1988). Roe deer are known as concentrate selectors, selecting plants or plant parts rich in easily digestible and highly nutritious cell contents such as proteins; they are limited in their ability to digest cellulose and fibre, structural components (Hofmann, 1985, 1988). Fallow deer are classified as intermediate feeders but err on the side of grass and roughage feeders (Figure 1.1, page 12). As well as being able to digest cell walls fallow deer are opportunistic feeders and are seasonally adaptive. The mouthparts of these species differ; fallow deer have a broader muzzle indicative of grazers and less selectivity and roe muzzles are narrower, and are more able to be selective. These intrinsic constraints interact with extrinsic ones, such as the nutritional quality of the food, toxicity etc.

The diet composition of fallow deer at both sites was lacking in the amount of grasses eaten when compared to published accounts. The deer at Denny Lodge ate more than those at Brinken. The reasons for this have been discussed earlier, namely the storm that made many plants more accessible and available to the deer. This highlights the opportunistic nature of the fallow deer. Their physiology is such that they can adapt to whatever they are presented with. Unfortunately not many individual grasses were chemically analysed, however the group Monocotyledon did not contain higher levels of nutrients than species considered to be taken opportunistically, *e.g.* Holly.

On first appearance the diet eaten by roe deer was not notably different to that of fallow, many of the plant species were common to both species deer. However they were eaten in different amounts and were thus different in their relative importance in the diet. It was apparent that by just investigating the three most commonly eaten plant species, roe deer, particularly at Squabb, took a much higher quality diet especially in terms of protein, calcium and magnesium. This was not simply due to the plants at Great Covert and Squabb containing more nutrients and the deer foraging more randomly. The nitrogen (protein) content in available plants was significantly higher at Great Covert but the deer at Squabb ate the highest quality diets. The plants at Squabb did produce the highest mean calcium content and the deer at this site ate a higher calcium diet than the others. For magnesium, there were similar concentrations available to both fallow and roe deer, but roe deer took a more magnesium rich diet by choice compared to fallow deer. All the deer took more than their requirement of potassium (Adam, 1988) and there was some doubt about the reliability of the phosphorus analysis. These data suggest that roe deer selected different plants or plant parts (in some cases) to fallow deer, such that they ate higher quality diets, especially in terms of magnesium, over several bimonths.

Unfortunately the number of roe deer available for feeding trials was limited. However there were differences in the protein content of the final diets both species of deer ate. In general, roe deer ate higher protein diets than fallow. The differences in digestive physiology, eating cell contents vs fibre, could not be considered in the trials nor could the bite size, although this may have been measured using the handling trials had sufficient roe been available. One of the few physical factors that could account for this difference was body size. The Bell-Jarman principle (see Section 1.3.3.3) is concerned with allometric scaling of metabolic rate in ruminants. It argues that small ruminants, such as roe deer, require high quality (low fibre) food to satisfy high metabolic rates as small ruminants have higher metabolic costs per unit volume than larger ones. This could explain why roe deer in the feeding trials took a higher protein diet than fallow deer. It also helps to explain why roe deer in their natural environment appeared to take a higher quality diet.

The differences in physiology and digestion were also indicated in the results from Trial Two (optimum protein vs high protein). All fallow deer fed Trial Two test diets developed an aversion to one or both diets, whereas roe deer ate both without any apparent aversion. Although it is unclear what this means and was only performed with three roe deer, it does hint that there are physiological differences between the two species.

7.2.4 Learning

Deer can be described as experienced or naive feeders depending on the food previously presented to them. Foraging in the familiar, natural environment will use knowledge acquired over time regarding the selection and consumption of plant species. Although there will be seasonal changes in quality and quantity these variations are likely to be within the limits of the deer's experience. However, even for experienced feeders it is important that they sample and resample vegetation particularly where toxins are involved, but this too will come with experience.

The data collected from fallow and roe deer in their natural environments were assumed to have measured diet selection of experienced individuals, faecal samples from young animals were not collected. Experienced feeders will have learnt about aversive and positive stimuli, and accept or reject foods accordingly. The stimuli experienced by the deer are pre-ingestive (tactile, olfactory, visual, gustatory) and post-ingestive (feedback post nutrient absorption). Although it was shown that, in general, both fallow and roe deer took diets that fulfilled their nutritional requirements, it was impossible to attribute this to any kind of learning. Nevertheless it is believed that the majority of choices made by these deer when selecting their diets were as a result of pre- and post-ingestive learning.

The feeding trials were designed to specifically look at the mechanisms of learning: whether or not fallow and roe deer could learn to differentiate between two diets that varied in protein content. The results from all the trials clearly indicated that both species of deer have the ability to learn and discriminate. Most of the park deer were normally fed concentrate food similar to that fed in the trials so from a tactile and visual perspective they were experienced, but in terms of the other sensory factors, both pre and post-ingestive, they were classed as naive. The trials were designed to force the deer to switch diets if learning took place and at most parks this was what happened. Moreover this switch took place after the first day suggesting that learning occurred as a result of post-ingestive feedback in the hours after absorption of the first day's intake. This supports findings by Provenza and Balph (1990). In addition, Arnold (1981) states that the perception of nutritional value is not generally immediate and cannot be recognised on consumption. Post-ingestive feedback could have been associated with certain stimuli (olfactory or gustatory) but not with others (tactile or visual) as both diets in a test were the same in these respects. The underlying mechanisms involved with learning in the park deer cannot be unravelled, however one of the feeding trials inadvertently brought about aversive learning in the form of conditioned food aversion. In this case, the aversive stimuli were hypothesised as being taste foremost and secondly smell, therefore it was likely that in the other trials where learning was indicated, the deer were using these stimuli coupled with positive post-ingestive consequences to select their diets. This may also help to explain why, in some cases, it took a few days to arrive at an optimum balance between the two diets, once the initial switch had been made.

There were two pre-ingestive processes that fallow and roe deer could have been using coupled with post-ingestive consequences, firstly trial and error and secondly social learning. The deer are likely to use trial and error in selecting and sampling novel foods in their environment and also in resampling plants known to vary in their palatability or toxicity. In the case of the feeding trials it was probable that they used this method, at least initially, on Day 1, coupled then with the intended 'pleasant' taste from one diet meant that they remained primarily with this diet for that whole session. Social behaviours have been shown to influence diet intake in the feeding trials, via conditioned food aversion, with the dominant male rounding up the group and subordinate animals closely following the dominant individuals. It is unclear as to whether or not these behaviours indicate a form of social learning. However, young animals have been shown to 'copy' what their conspecifics eat (Provenza and Balph, 1991), and avoid what is not eaten (Provenza and Balph, 1987, 1988; Thorhallsdottir *et al*, 1987). Social learning also occurs in the form of communication through cues such as olfaction (*e.g.* Galef *et al*, 1984). The study deer were not followed after they had fed on the diets, but as they are a social species and often come togther to ruminate in small groups, there may have been some communication about the diets at this time. This is, however very speculative as the only evidence for this exists for rats (Galef and Heiber, 1976; Galef and Wigmore, 1983; Galef *et al*, 1984) and not for ruminants.

7.2.5 Optimal foraging

Optimal foraging theories were not considered in the design of this thesis, however they incorporate many of the models (mechanisms) already discussed such as euphagia (*e.g.* sodium, Belovsky, 1978, 1981) and certainly learning (Kamil and Roitblat, 1985). From the review by Stephens and Krebs (1986) the literature suggests that rate maximisation models, subject to constraints, best explain diet selection in herbivores. Illius and Gordon (1993) refine this, saying nutrient and energy intake subject to avoiding harmful chemicals (*e.g.* toxins and poisons) is the most likely explanation in large herbivores. However, there is very little evidence to suggest that herbivores select nutrients to balance their diets (Stephens and Krebs, 1986). Illius and Gordon (1993) believe deer employ strategies to achieve this aim but are constrained by discriminating between foods of different nutritional content. This discrimination brings in some of the models discussed earlier. It is usually assumed that only a complex diet will meet an animal's requirements but ruminants can make many amino acids and many other essential nutrients (Owen-Smith and Novellie, 1982). In addition, by selecting certain plants or plant parts to maximise one nutrient, they may maximise the intake of many or all nutrients at the same time. This study showed that diet composition does not have to be complex, as by selecting the three most commonly eaten plant species in a bimonth, fallow and roe deer generally fulfilled their nutritional requirements.

7.2.6 Conclusions

The concept of learning has only recently been suggested as having a role in diet selection. It has been argued that it is an innate ability and that its importance and use has been heightened as a result of natural selection in changing foraging environments. This thesis was designed to investigate, diet selection in fallow and roe deer, firstly by looking at what they selected in their natural environment, why and to some extent how. Secondly it investigated deer's learning abilities through feeding trials. Learning was clearly shown in both species of deer and although other models of diet selection, including hedyphagia, morphophysiology, optimal foraging, come into play in the natural habitat, learning was evidently fundamental to the diet selection processes of fallow and roe deer. Illius and

Gordon (1987, 1991) stated that the mechanisms of diet selection in red deer are the function of two properties of the vegetation, quantity (availability) and quality which both interact with the animal's size. This thesis has shown that the quality of vegetation coupled with its availability in the local habitat were involved in the mechanisms of diet selection, as were the deer's size and morphophysiology, but it has also shown that learning was almost certainly an integral part of the selection process.

7.3 APPLICATION FOR MANAGEMENT

The results from this study could be beneficial in two ways; for the deer themselves and also for the people that manage them. Accounts of what fallow and roe deer eat at different times of the year and why, in terms of nutrients, can be drawn up from this study:-

Winter:	fallow deer preferred to eat Pine, Holly and Moss roe deer preferred to eat Western hemlock, Bramble and Ivy
Spring / Summer:	fallow deer preferred to eat Oak and Monocotyledons roe deer preferred to eat Oak and Monocotyledons

These plant species fulfilled all the nutrient requirements (crude protein, calcium, magnesium and potassium) for the respective time of year. Compared to fallow deer, roe deer were associated more with vegetation of higher crude protein content particularly in the winter. This apparent higher winter protein requirement of roe deer over fallow deer was also found in the feeding trials. It was expected that male deer, would prefer vegetation high in calcium when their antlers were regrowing, particularly fallow deer as they possess much larger antlers than roe deer. Fallow and roe deer grow their antlers at different times of the year; fallow deer in MayJun and roe deer in JanFeb. Fallow deer did not select a calcium rich diet to assist antler growth, this was largely due to plant calcium levels being at their lowest at this time. Male roe deer, however have the advantage of growing their antlers at the time of peak calcium concentration in plants and were observed to select a more calcium rich diet than fallow deer.

These findings could be used to help with deer management issues, in parks and in the wild such as those found on Forestry Commission property, conservation areas and on farmland. In a parkland setting, specific vegetation could be grown and cultivated that the deer prefer but that also provide them with the most available nutrients. This will ensure healthy deer, better able to survive the winter, to reproduce successfully, to withstand parasites etc., which will also produce good carcass weights if culled for venison.

Farmers/land owners/keepers could grow specific preferred plants for the deer which may in turn stop the deer taking vegetation that the land owners/keepers do not want them to eat, this has been described as 'diversionary feeding' by Putman (1998). This is particularly a problem with roe deer on arable land (Putman, 1986b), intercropping with more palatable forage has been suggested (Putman, 1998). It may even be possible to attract these deer back into the woodland by careful management of what is planted and grown. For example, managing woodland to contain Bramble, a preferred plant species of roe deer found in this study and others, may encourage the deer away from crops, however it is likely that more than just Bramble would have to be on offer. Putman (1998) reports the enlargement of woodland clearings and reseeding using earlier maturing grasses actually took roe deer away from arable fields in significant numbers. It may also be possible to offer artificial foods in place of naturally preferred forages, *e.g.* almond flavouring was found to be particularly favoured by fallow deer in the current study.

Changing the patterns of habitat use or altering foraging behaviour may only be beneficial in the short term unless carefully considered and implemented. It would also rely on the kind of environment requiring protection from deer. The disadvantage for the farmer or landowner may be that managing their crop becomes more difficult and expensive, *e.g* in terms of machinery. Also these measures may actually encourage more deer into the area, or increase the fecundity such that they spill out into the arable areas once more to sustain the increased numbers. So, although this study suggests management implications they would require careful thought and piloting.

7.4 FURTHER WORK

This study has answered fully the research questions put forward, however it has also brought to light many more questions of interest. Diet selection is extremely complicated and is dependent on many factors, the current work has attempted to unravel some of these factors but in a very simplistic manner. To gain a clearer understanding there must be more control in the collection of data. Standardised controlled feeding trials are only part of the answer. Complete chemical profiles of the plants eaten should include levels of toxins and poisons. The effects of these chemicals on selection at different times of year should be monitored. Availability is still believed to be the cornerstone of diet selection therefore more detailed and comprehensive measures should be taken. Further feeding trials should be carried out to initially observe whether the deer always arrive at the same preference score irrespective of the concentrations in the diets. They should strive to be more controlled, *i.e.* removing the possibility of eating foods other than the test diets or retrieving the diets after half had been eaten in accordance with preference testing. Having uncovered conditioned food aversion this would be an interesting area to pursue particularly when coupled with flavouring and especially in comparing fallow and roe deer. The current study was pioneering, so many of these additional suggestions were too time consuming or impractical and had they been brought into place may have resulted in measuring something very different to what was intended. This thesis has certainly paved the way for stimulating future research into diet selection of fallow and roe deer.

Appendices

APPENDIX 1

List of plants found in the diet of both fallow and roe deer, common names and scientific names given.

Common Name	Scientific Name
Conifer Trees and Evergreens	
Douglas Fir	*Pseudotsuga menziesii*
European Larch (deciduous leaves)	*Larix decidua*
Holly	*Ilex aquifolium*
Ivy	*Hedera helix*
Lodgepole Pine	*Pinus contorta*
Norway Spruce	*Picea abies*
Rhododendron	*Rhododendron ponticum*
Scot's Pine	*Pinus silvestris*
Western Hemlock	*Tsuga heterophylla*
Yew	*Taxas baccata*
Deciduous trees	
Alder	*Alnus glutinosa*
Ash	*Fraxinus excelsior*
Beech	*Fagus sylvatica*
Elder	*Sambucus nigra*
Hawthorn	*Crataegus monogyna*
Hazel	*Corylus avellana*
Oak	*Quercus robur*
Pussy Willow	*Salix caprea*
Silver Birch	*Betula pendula*
Sweet Chestnut	*Castanea sativa*
Sycamore	*Acer pseudoplatanus*

Common Name	Scientific Name	
Grasses, Rushes and Sedges		
Annual Meadow Grass	*Poa annua*	Sw
Bristle-Leaved Bent	*Agrostis setacea (curtisii)*	C
Chalk False Brome	*Brachypodium pinnatum*	C
Cocksfoot	*Dactylis glomerata*	C
Common Bent	*Agrostis tenuis (capillaris)*	Sw
Creeping Bent	*Agrostis stolonifera*	C
Heath Grass	*Sieglingia decumbens*	C
Perennial Rye-Grass	*Lolium perenne*	Sw
Purple Moor Grass	*Molinia caerulea*	C
Smooth Meadow Grass	*Poa pratensis*	Sw
Strong Creeping Red Fescue	*Festuca rubra*	Sw
Tufted Hair-Grass	*Deschampsia caespitosa*	C
Upright Brome	*Bromus erectus*	C
Velvet Bent	*Agrostis canina*	Sw
Wood False Brome	*Brachypodium sylvaticum*	C
Wavy Hair-Grass	*Deschampsia flexuosa*	C
Yorkshire Fog	*Holcus lanatus*	Sw
Cereal - Barley	*Hordeum vulgare*	
Cereal - Wheat	*Triticum aestivum*	
Sedges (True)	*Carex* spp.	
Rushes	*Juncus* spp.	

Common Name	Scientific Name
Shrubs	
Dog Rose	*Rosa canina*
Bramble	*Rubus fruticosus*
Honeysuckle	*Lonicera periclymenum*
Ferns	
Bracken	*Pteridium aquilinum*
Hard Fern	*Blechnum spicant*
Male Fern	*Dryopteris pseudomas*
Mosses	
	Cirriphyllum piliferum
	Dicranum scoparium
	Hypnum cupressiforme
	Mnium hornum
	Polytrichum spp.
	Sphagnum spp.
	Thuidium tamariscinum
Dwarf shrubs	
Bell Heather	*Erica cinerea*
Bilberry	*Vaccinium myrtillus*
Bog Heather	*Erica tetralix*
Bog Myrtle	*Myrica gale*
Broom	*Cytisus scoparius*
Dwarf gorse	*Ulex minor*
Gorse	*Ulex europea*

Common Name	Scientific Name
Ling	*Calluna vulgaris*
Herbs	
Birds Foot Trefoil	*Lotus corniculatus*
Bluebell	*Endymion non-scriptus*
Bugle	*Ajuga repans*
Buttercup	*Ranunculus acris*
Common Dog Violet	*Viola riviniana*
Common Speedwell	*Veronica officinalis*
Creeping Buttercup	*Ranunculus repens*
Daisy	*Bellis perennis*
Dandelion	*Taraxacum officinale*
Foxglove	*Digitalis purpurea*
Germander Speedwell	*Veronica chamaedrys*
Ground Ivy	*Glechoma hederacea*
Heath Bedstraw	*Galium saxatile*
Lesser Celandine	*Ranunculus ficaria*
Ragwort	*Senecio jacobaea*
Rape	*Brassica napus*
Selfheal	*Prunella vulgaris*
Spear Thistle	*Cirsium vulgare*
Tormentil	*Potentilla erecta*
White clover	*Trifolium repens*
Wood Anemone	*Anemone nemorosa*
Wood Sorrel	*Oxalis acetosella*

Legend: Sw = sweet grasses C = coarse grasses taken from Putman *et al* (1993)

APPENDIX 2

Plant Groupings

F = used in the faecal analysis where it was not possible to classify a potentially identifiable plant fragment down to species level, it was therefore classified within its group

C = used for grouping plant species in order to compare the diet of fallow and roe deer with published data

Group	Usage	Definition
Deciduous tree	FC F	all trees that are deciduous, *i.e.* cast their leaves annually (see Appendix 1 for complete list). plant fragment discernable as being a dicotyledon but moreover a deciduous tree due to the thicker, more angular cell walls.
Dicotyledon [1]	F	plant fragment discernable only as being a dicotyledon as opposed to a monocotyledon due to lack of long, narrow cells.
Monocotyledon [2]	FC F	all grasses, sedges and rushes (see Appendix 1 for complete list). plant fragment discernable only as being a monocotyledon as opposed to being a dicotyledon.
Coarse grass [3]	F	plant fragment discernable as being a monocotyledon but moreover a grass that contains many fibres, has thicker cell walls and many silica bodies.
Sweet grass [4]	F	plant fragment discernable as being a monocotyledon but moreover a grass that is not coarse, has few fibres and thinner cell walls and few silica bodies.
Conifers	C	Any of various mostly needle-leaved or scale-leaved, chiefly evergreen, cone-bearing gymnospermous trees or shrubs such as pines, spruces, and firs. (see Appendix 1 for complete list).
Dwarf shrubs	C	A woody plant of relatively low height, having several stems arising from the base and lacking a single trunk; a bush. Includes heathers, gorses etc (see Appendix 1 for complete list).
Ferns	C	Any of numerous flowerless, seedless vascular plants having roots, stems, and fronds and reproducing by spores. Pteridopytes (see Appendix 1 for complete list).

Herbs	FC	A plant whose stem does not become woody and permanent, but dies, at least down to the ground, after flowering. *Note:* Annual herbs live but one season; biennial herbs flower the second season, and then die; perennial herbs produce new stems year after year. (see Appendix 1 for complete list).
Moss	FC	*Polytrichum* spp. (major component) and *Sphagnum* spp. were identifiable to species level, other mosses (few) were grouped and incorporated into the Moss group. (see Appendix 1 for complete list).
Unknown	F	plant fragment unidentifiable as being a monocotyledon or a dicotyledon but is certainly a plant fragment.

[1] A plant whose seeds divide into two seed lobes, or cotyledons, in germinating.

[2] Any of various flowering plants, in this case grasses, having a single cotyledon in the seed.

[3] and [4] see Appendix 1

APPENDIX 3

Frequency of occurrence of plant species (and groups) found at each site.

(legend: shaded rows are those species or groups that **do not** fulfil the criteria for inclusion: ≥ 2 bimonths **and** ≥ 2 occurrences)

Table A3.1 Brinken

	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Alder	0	1	2	0	0	0
Agrostis canina	0	0	1	0	0	0
Agrostis setacea	0	2	13	19	19	5
Agrostis stolonifera	0	0	0	4	0	0
Agrostis tenuis	0	4	3	0	0	0
Beech	0	6	8	13	6	0
Birch	0	3	16	19	14	0
Blechnum spicant	0	0	0	0	0	3
Bog Asphodel	0	9	7	6	4	6
Brachypodium pinnatum	0	0	0	2	1	0
Brachypodium sylvaticum	0	10	18	20	20	16
Bracken	2	6	5	15	19	5
Bramble	14	17	16	19	20	18
Bromus erectus	0	6	7	12	9	6
Broom	7	12	13	9	6	10
Calluna	9	5	0	2	2	5
Carex spp.	5	11	13	17	18	13
Coarse grass	0	0	4	7	1	0
Coarse monocotyledon	0	9	13	20	12	8
Dactylis glomerulata	7	19	15	7	20	18
Deciduous tree	16	15	19	20	19	18
Deschampsia caespitosa	0	0	6	10	2	0
Deschampsia flexuosa	1	0	8	0	19	14
Dicotyledon	16	19	19	20	20	18

Dwarf gorse	17	11	8	2	6	0
Elder	0	5	6	2	0	0
Erica cinerea	6	0	1	10	3	3
Festuca rubra	0	2	6	15	8	1
Herb	0	11	17	17	11	1
Grass	5	6	16	20	20	15
Ground ivy	0	0	2	0	0	0
Hawthorn	0	2	11	7	2	0
Holcus lanatus	0	4	8	0	6	3
Holly	20	20	14	20	18	18
Ivy	19	20	17	16	14	16
Juncus spp.	16	14	8	12	14	7
Lolium perenne	0	11	11	20	13	5
Lonicera periclymenum	3	7	0	0	5	2
Molinia caerula	0	0	12	9	17	5
Monocotyledon	10	16	19	20	20	18
Moss	20	20	18	20	20	18
Norway Spruce	1	2	0	0	0	0
Oak	6	10	18	20	20	18
Pine	15	12	5	12	11	16
Poa annua	0	2	1	0	0	0
Poa pratensis	0	0	1	5	0	0
Sieglingia decumbens	0	9	16	19	12	6
Sparganium erect.	0	7	0	0	0	0
Sphagnum	0	3	4	12	11	1
Thistle	0	2	8	13	3	0
Vaccinium myrtillus	11	9	9	5	4	1
Western hemlock	0	0	2	0	0	0
Wood spurge	4	2	0	0	0	0
Yew	0	0	5	0	3	2
Sample size (N)	**20**	**20**	**19**	**20**	**20**	**18**

Table A3.2 Denny Lodge

	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Agrostis sp.	0	0	9	19	5	0
Agrostis canina	0	0	3	10	0	0
Agrostis setacea	0	2	8	18	14	7
Agrostis stolonifera	0	0	2	7	0	0
Agrostis tenuis	0	0	0	5	0	2
Bark	2	0	0	0	0	11
Beech	0	7	6	8	3	0
Birch	0	2	9	6	12	10
Bog Asphodel	3	0	0	0	6	2
Brachypodium sylvaticum	2	0	8	8	9	8
Bracken	2	14	3	12	14	12
Bramble	9	4	0	5	7	8
Bromus erectus	0	3	8	9	10	11
Broom	1	5	0	0	0	0
Calluna vulgaris	12	10	0	0	3	10
Carex spp.	10	9	15	20	20	19
Coarse grass	0	4	15	19	6	5
Coarse monocotyledon	0	16	19	20	20	19
Dactylis glomerulata	2	2	6	3	9	15
Deciduous tree	6	10	20	20	20	20
Deschampsia caespitosa	2	0	9	16	4	1
Deschampsia flexuosa	0	0	0	3	4	0
Dicotyledon	20	20	20	20	20	20
Dryopteris pseudomas	0	0	0	0	0	2
Dwarf gorse	0	3	0	0	0	0
Erica cinerea	8	6	2	6	5	8
Festuca rubra	0	3	9	5	2	2
Herb	6	0	8	19	19	14
Grass	3	16	19	20	20	15
Ground ivy	0	4	0	4	2	3

Holcus lanatus	0	2	2	5	2	3
Holly	18	16	3	0	9	9
Ivy	17	16	13	6	12	13
Juncus spp.	20	20	12	17	14	20
Lolium perenne	0	0	0	5	2	8
Lonicera periclymenum	8	11	3	0	15	8
Molinia caerula	0	0	8	5	0	4
Monocotyledon	17	20	20	20	20	20
Moss	20	20	18	20	20	20
Nardus stricta	0	0	0	4	0	0
Norway Spruce	0	0	2	0	2	2
Oak	0	2	12	19	19	18
Pine	19	20	18	18	20	20
Poa annua	0	2	2	0	0	0
Sieglingia decumbens	2	0	16	12	13	13
Sparganium erect.	0	0	0	0	2	0
Sphagnum	1	2	6	5	6	0
Thistle	1	6	1	1	3	0
Vaccinium myrtillus	5	0	0	0	9	3
Western hemlock	0	1	4	1	11	11
Wood spurge	0	3	0	2	2	5
Yew	2	0	2	0	5	0
Sample size (N)	**20**	**20**	**20**	**20**	**20**	**20**

Table A3.3 Great Covert

	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Alder	0	0	2	5	8	9
Agrostis canina	0	0	2	2	0	0
Agrostis setacea	0	0	3	4	0	0
Beech	0	0	0	0	0	1
Birch	0	0	6	6	11	1
Blechnum spicant	9	2	0	5	14	11
Brachypodium sylvaticum	0	5	7	7	3	2
Bracken	5	2	0	9	10	4
Bramble	8	10	10	18	17	17
Calluna vulgaris	0	0	0	3	0	0
Carex spp.	2	8	9	9	9	5
Cereal	8	11	9	0	0	0
Dactylis glomerulata	16	17	11	2	4	13
Dandelion	0	6	2	0	0	0
Deciduous tree	6	5	14	18	17	20
Deschampsia flexuosa	0	0	4	6	4	1
Dicotyledon	18	19	15	18	17	20
Douglas fir	20	20	12	11	15	17
Dryopteris pseudomas	7	1	0	0	7	9
Elder	0	1	0	5	0	0
Herb	8	13	14	17	13	11
Grass	0	4	2	7	3	2
Hawthorn	0	0	1	0	1	0
Holcus lanatus	1	6	2	2	0	1
Holly	0	0	0	0	4	0
Ivy	15	18	2	6	4	8
Juncus spp.	3	6	8	8	7	3
Larch	0	0	2	3	0	0
Lolium perenne	10	7	2	0	0	5
Lonicera periclymenum	0	0	0	0	2	0

Lotus spp.	0	5	2	1	8	1
Molinia caerula	0	0	0	2	0	0
Monocotyledon	18	19	15	18	17	17
Moss	5	12	14	18	13	11
Oak	3	0	6	13	15	16
Pine	18	12	9	3	12	18
Poa pratensis	0	0	0	1	0	0
Privet	0	0	0	0	0	3
Ragwort	0	0	0	0	4	3
Rumex acetosa	0	0	0	0	2	0
Sieglingia decumbens	0	1	2	9	0	0
Sweet chestnut	2	0	1	2	1	6
Thistle	0	0	4	2	0	0
Western hemlock	20	20	15	15	17	20
White clover	0	2	4	11	2	0
Wood spurge	0	0	0	0	4	0
Yew	0	0	0	1	2	0
Sample size (N)	**20**	**20**	**15**	**18**	**17**	**20**

Table A3.4 Squabb

	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Alder	0	0	5	9	14	6
Ash	0	0	1	5	5	1
Agrostis setacea	0	0	3	0	3	0
Agrostis tenuis	0	1	0	0	0	0
Beech	0	0	8	3	4	0
Birch	1	3	19	7	14	2
Blechnum spicant	13	0	2	0	0	12
Bog Asphodel	0	0	0	2	0	0
Brachypodium sylvaticum	0	1	0	0	0	0
Bracken	13	0	12	1	2	13
Bramble	20	19	20	14	17	15
Bromus erectus	1	0	0	0	0	0
Broom	0	0	2	1	0	0
Calluna vulgaris	0	0	0	0	0	1
Carex spp.	19	16	14	13	17	15
Cereal	4	9	1	0	2	0
Dactylis glomerulata	9	11	5	0	2	2
Dandelion	0	0	0	4	0	0
Deciduous tree	20	17	18	14	17	15
Deschampsia flexuosa	1	2	1	0	3	1
Dicot	18	18	19	14	17	15
Douglas fir	0	0	0	8	4	0
Dryopteris pseudomas	2	0	0	0	0	9
Elder	0	1	2	5	3	0
Festuca rubra	0	0	1	0	1	0
Herb	0	0	11	12	8	0
Gorse	12	11	9	3	15	15
Grass	2	2	6	1	6	0
Hawthorn	0	0	0	0	7	0
Hazel	0	0	8	5	1	0

Holcus lanatus	2	5	4	1	5	0
Holly	0	0	2	1	0	0
Ivy	18	19	16	12	17	15
Juncus spp.	0	3	2	11	3	1
Larch	0	2	4	12	0	0
Lolium perenne	2	3	1	0	0	0
Lonicera periclymenum	0	1	0	0	3	6
Molinia caerula	0	1	1	0	0	0
Monocotyledon	15	18	15	13	16	14
Moss	13	12	7	10	15	11
Nardus stricta	0	0	0	2	0	0
Oak	6	2	11	11	16	14
Pine	18	17	13	7	1	5
Privet	0	3	0	3	6	4
Ragwort	0	0	2	2	0	0
Rape	6	0	0	0	0	5
Rumex acetosa	0	0	1	0	2	0
Sieglingia decumbens	0	0	1	0	0	0
Sparganium erect.	0	0	1	0	0	0
Sweet chestnut	1	2	1	0	8	0
Sycamore	0	0	0	1	0	0
Thistle	2	3	6	0	2	4
Western hemlock	5	10	8	5	0	6
White clover	0	0	0	2	0	0
Willow	4	2	4	4	16	6
Yew	2	5	6	6	0	0
Sample size (N)	**20**	**19**	**20**	**14**	**17**	**15**

Appendix 4

Main dietary components of the diets at each site, measured as mean proportions of the entire diet, determined using faecal analysis.

Legend:

no shading = ≥5% of diet in any one bimonth;
light shading plus no shading = ≥2% in any one bimonth;
dark shading = ≥1% but <2% in any one bimonth

Table A4.1 Brinken

Plant	JanFeb	MarApr	MayJun	JulAug	SepOct	NovDec
Holly	55.64	32.97	5.3	5.72	11.02	31.37
Moss	18.89	22.67	11.92	15.04	13.65	12.49
Oak	0.217	0.392	18.68	13.67	6.93	4.54
Bramble	0.90	2.59	2.03	6.63	10.84	11.62
Ivy	4.37	8.21	4.91	2.79	2.05	2.27
Brachypodium sylvaticum	0.02	0.43	2.34	4.9	6.8	1.49
Pine	6.2	3.43	0.22	1.14	0.85	2.96
Deciduous tree	0.82	4.14	7.78	8.77	6.62	7.49
Monocotyledon	0.27	3.02	7.05	4.3	5.38	5.16
Dicotyledon	1.27	5.24	7.35	5.2	4.93	4.49
Dactylis glomerata	0.18	3.04	2.02	0.17	4.56	5.1
Agrostis setacea	0.02	0.09	2.55	4.96	2.46	0.19
Sieglingia decumbens	0.03	0.9	2.85	2.81	0.87	0.89
Bracken	0.05	0.32	0.61	2.52	4.16	0.41
Carex spp.	0.12	0.52	1.02	1.44	2.68	0.93
Molinia caerulea	0	0	1.76	0.64	3.52	0.41
Birch	0	0.21	1.84	2.9	0.67	0
Juncus spp.	3.44	1.59	0.3	0.49	0.65	0.5
Deschampsia flexuosa	0.05	0	0.35	0.02	3.12	1.55
Dwarf gorse	3.41	0.39	0.57	0.03	0.21	0

Lolium perenne	0	0.67	0.71	2.04	0.56	0.21
Coarse grass	0	0.36	2.12	2.92	0.77	0.35
Grass	0.1	0.36	2.02	2.67	2.3	1.81
Broom	0.32	0.8	1.94	0.65	0.24	0.91
Calluna vulgaris	1.97	0.34	0.02	0.05	0.03	0.35
Lonicera periclymenum	0.22	1.64	0.04	0	0.10	0.04
Unknown	0.18	0.63	1.45	0.9	1.14	0.61

Table A4.2 Denny Lodge

Plant	**JanFeb**	**MarApr**	**MayJun**	**JulAug**	**SepOct**	**NovDec**
Pine	26.43	35.57	7.5	4.06	24.35	35.21
Moss	42.42	29.22	9.89	10.62	13.27	15.51
Oak	0	0.05	9.47	2.95	5.35	3.45
Holly	10.93	1.77	0.23	0.02	0.73	0.34
Carex spp.	0.33	0.21	1.09	3.21	4.69	3.43
Juncus spp.	6.22	3.45	0.52	1.12	1.2	2.27
Deciduous tree	0.13	2.92	7.86	6.33	12.02	12.06
Monocotyledon	0.98	6.17	21.63	17.34	7.11	4.14
Coarse grass	0.25	1.44	16.6	18.36	3.45	2.49
Grass	0.18	0.61	7.16	8.59	2.6	0.9
Dicotyledon	2.87	5.33	4.21	4.86	7.68	4.79
Herb	0.18	0.02	0.46	7.14	2.42	0.8
Ivy	4.59	1.31	1.76	0.27	1.05	1.35
Agrostis spp.	0	0	1.17	3.44	0.14	0.02
Agrostis setacea	0	0.04	0.59	2.49	0.61	0.19
Calluna vulgaris	1..52	4.88	0.02	0	0.31	1.57
Bracken	0.2	0.53	0.08	1.24	3.0	0.71
Sieglingia decumbens	0.07	0.04	1.63	0.65	0.84	0.86
Birch	0.02	0.14	0.36	0.92	1.88	0.64
Erica cinerea	0.32	1.03	0.1	0.13	0.23	1.57
Western hemlock	0	0.04	1.93	0.07	0.52	0.81

Lonicera periclymenum	0.42	0.79	0.13	0	1.45	0.58
Deschampsia caespitosa	0.05	0	0.84	1.49	0.05	0.07
Dactylis glomerata	0.03	0.04	0.21	0.12	0.3	1.2
Beech	0	1.03	0.25	0.17	0.09	0.02
Vaccinium myrtillus	0.17	0	0	0	1.03	0.15
Dwarf shrub	0	1.07	0	0	0	0
Unknown	0.32	1.21	1.26	1.27	1.57	1.49

Table A4.3 Great Covert

Plant	**JanFeb**	**MarApr**	**MayJun**	**JulAug**	**SepOct**	**NovDec**
Western hemlock	68.43	59.02	18.6	15.61	37.67	53.88
Douglas fir	11.21	15.71	2.32	2.91	5.57	8.24
Bramble	0.99	1.14	1.12	17.46	8.69	4.78
Oak	0.07	0	9.8	3.89	4.06	6.81
Ivy	5.86	3.83	0.68	2.31	1.32	2.37
Cereal	0.64	1.76	7.35	0	0	0
Deciduous tree	0.17	0.53	7.63	11.05	10.92	5.42
Monocotyledon	3.44	5.27	12.61	8.96	3.34	3.07
Dicotyledon	1.39	2.07	4.93	5.78	4.89	2.87
Herb	0.22	1.64	12.61	8.96	4.25	1.07
Pine	2.46	1.22	0.87	0.25	2.23	1.85
Dactylis glomerata	2.17	3.08	1.17	0.06	0.23	1.81
Polytrichum spp.	0.13	0.75	2.59	2.74	1.13	0.42
Bracken	0.17	0.08	0.05	4.55	2.59	0.19
Blechnum	0.43	0.24	0	0.25	3.1	3.15
Calluna vulgaris	0	0.04	0.03	4.86	0	0
Carex spp.	0.05	0.28	1.55	0.64	2.17	0.15
Lolium perenne	1.45	0.69	0.16	0	0	0.92
Birch	0	0	0.87	0.85	1.23	0.07
White clover	0	0.06	0.55	1.42	0.06	0.02
Ragwort	0	0	0	0.02	1.36	0.34

Unknown	0.27	0.38	1.31	1.64	1.02	0.34

Table A4.4 **Squabb**

Plant	**JanFeb**	**MarApr**	**MayJun**	**JulAug**	**SepOct**	**NovDec**
Bramble	56.37	49.0	30.15	30.77	27.32	48.06
Ivy	9.96	12.55	6.0	7.84	11.57	13.96
Oak	0.49	0.08	10.48	8.48	17.16	3.19
Pine	11.98	11.96	2.03	0.29	0.11	3.33
Birch	0.27	0.13	11.55	1.38	3.93	0.05
Bracken	0.82	0.03	3.82	0.06	2.94	5.71
Juncus spp.	0	0.11	0.06	5.48	0.14	0.05
Polytrichum spp.	0.77	0.61	0.64	5.17	1.19	0.83
Deciduous tree	6.07	3.62	7.03	10.87	14.61	7.72
Monocotyledon	2.49	3.78	5.56	3.03	2.4	1.32
Larch	0	0.08	0.29	4.04	0.02	0
Carex spp.	2.49	1.98	2.58	3.09	3.51	3.44
Gorse	0.94	0.79	0.98	2.05	2.17	1.39
Alder	0.02	0	0.38	2.65	1.71	0.35
Holcus lanatus	0.04	2.91	1.16	0.09	0.27	0.05
Western hemlock	0.44	2.35	1.39	0.26	0.05	0.51
Willow	0.1	0.11	0.29	0.26	2.21	0.28
Dicotyledon	1.65	2.25	4.89	4.64	3.29	2.75
Herb	0	0	1.48	2.08	0.55	0.02
Dactylis glomerata	1.23	1.98	1.01	0	0.18	0.42
Blechnum spicant	0.64	0.05	0.09	0	0.02	4.02
Yew	1.03	1.27	0.38	0.78	0	0
Hazel	0	0	1.74	1.21	0.05	0
Cereal	0.32	1.85	0.23	0	0.11	0.02
Beech	0	0	1.04	0.52	0.16	0
Dryopteris pseudomas	0.04	0	0.04	0	0	1.23
Unknown	0.33	0.24	0.52	1.38	0.43	0.07

APPENDIX 5

The availability of plant species in summer and winter at each of the four sites, measured as a proportion of the total samples. Also included, the volume and occurrence of species data as used in the statistical analysis. Plant species included in the volume data are those that occurred at ≥1% of diet in any of the six original bimonths and for the occurrence data these were plant species with at least two occurrences in at least two samples (*i.e.* two deer) and occurring in at least two bimonths over the year.

Legend:

† = Herb (included in availability analysis collectively)

- = missing data (species highly likely to be present as they were found in the other season, but were not recorded as eaten either in terms of volume or occurrence in diet)

Moss (other) not included in analysis as Moss recorded in faecal analysis was predominantly *Polytrichum* spp.

Table A5.1 Brinken

Vegetation	Summer			Winter		
	Proportion available	Proportion eaten	Proportion occur	Proportion available	Proportion eaten	Proportion occur
Bramble	13.75	3.35	94.74	13.33	6.96	100
Holly	12.5	2.66	73.68	17.92	37.23	100
Bracken	10.83	0.77	-	5	0.03	10
Calluna vulgaris	9.58	-	-	13.75	1.15	40
Agrostis stolonifera	9.17		10.53	3.75		
Molinia caerula	7.08	1.41	52.63	0.83	0.35	25
Holcus lanatus	4.58		15.79	0.42		15
Agrostis tenuis	4.58		10.53	9.17		-
Carex spp.	3.75	1.17	78.95	3.33	0.7	55
Herb	3.75	1.09	84.21	2.5	0.03	5
Tormentil †	3.75			0		
Sieglingia decumbens	2.92	2.7	94.74	1.67	0.82	30
Oak	2.08	24.04	100	0	2.57	70
Polytrichum spp.	2.08	12.7	94.74	15	20.93	100

Moss (other)	1.25			6.67		
Heath Bedstraw	1.67			0.42		
Ivy	1.25	2.06	84.21	0.83	2.89	90
Hawthorn	1.25		57.89	0		0
Common Dog Violet †	1.25			0		
Bristly ox-tongue	1.25			0.42		
Silver birch	1.25	2.2	94.74	0	0	0
Dwarf gorse	0.83	0.11	26.32	0	1.99	45
Honeysuckle	0.83	0.02	-	0	0.25	25
Dactylis glomerata	0.42	0.35	21.05	0.42	3.37	70
Foxglove †	0.42			0.73		
Lesser celandine †	0.42			0		
Butchers broom	0.42			0		
Deschampsia flexuosa	0	0.19	21.05	0.42	1.09	45
Dead leaves	0			3.75		
Daisy †	0			0.83		
Common speedwell †	0			0.42		

Table A5.2 Denny Lodge

Vegetation	Summer			Winter		
	Proportion available	**Proportion eaten**	**Proportion occur**	**Proportion available**	**Proportion eaten**	**Proportion occur**
Beech	12.12	0.2	45			
Polytrichum spp.	12.12	11.65	95	20.08	25.14	100
Moss (other)	10.23			12.88		
Bracken	9.85	0.53	25	3.79	0.54	35
Juncus spp.	6.06	0.8	70	6.82	4.14	100
Agrostis tenuis	4.92		10	6.06		-
Silver birch	4.92	0.7	45	0	0.47	30
Douglas fir	4.17			5.3		
Holly	3.41	0.02	-	3.79	8.13	75

Herb	3.41	3.79	80	1.5	0.37	50
Western hemlock	3.03	0.2	10	4.55	0.22	25
Cedar	3.03			4.17		
Deschampsia caespitosa	3.03	1.53	75	3.79	0.05	10
Ivy	2.65	3.69	35	3.03	3.69	75
Holcus lanatus	2.27		20	3.41		15
Bramble	2.27		15	3.41		35
Molinia caerula	2.27		45	1.89		20
Brachypodium sylvaticum	2.27		40	3.03		35
Oak	1.52	9.81	100	0	2.29	50
Trefoil †	0.76			0		
Honeysuckle	0.76	0	-	0.76	0.58	60
Wood sorrel †	0.76			0		
Carex spp.	0.28	2.47	90	1.14	2.08	85
Buttercup †	0.38			0		
Daisy †	0.38			0.38		
Calluna	0.38	0	-	1.14	0.58	55
Agrostis setacea	0.38	1.58	75	0.38	0.03	5
Lolium perenne	0.38		20	0.76		20
Bristly ox-tongue	0.38			0		
Heath Bedstraw	0.38			0.76		
Poa annua	0.38			0		
Agrostis canina	0.38		40	0.76		-
Sieglingia decumbens	0.38	1.18	70	0.76	0.54	45
Festuca rubra	0.38		30	0		10
Agrostis stolonifera	0.38		15	0		-
European Larch	0.38			0		
Dead leaves	0			5.30		
Pine	0	3.39	85	1.52	32.02	100
Foxglove †	0			0.38		

Table A5.3 Great Covert

Vegetation	Summer			Winter		
	Proportion available	Proportion eaten	Proportion occur	Proportion available	Proportion eaten	Proportion occur
Western hemlock	31.25	8.38	93.33	33.75	59.79	100
Douglas fir	22.08	1.41	46.67	23.33	9.8	85
Silver birch	6.25	1.78	80			
Bramble	5	6.4	86.67	7.5	0.75	50
Moss (other)	4.17			2.5		
Bracken	3.75	5.35	46.67	0.83	0.19	20
Herb	3.75	20	100	1.67	0.48	50
Ivy	5	0.07	13.33	10.83	3.54	50
Oak	2.08	11.15	73.33	0	4.3	50
Agrostis tenuis	2.92			2.08		
Juncus spp	1.67		53.33	1.25		30
Thistle	1.67		13.33	2.5		-
Polytrichum spp.	1.25	3.08	100	4.17	0.23	30
Beech	1.25			0		
Holcus lanatus	1.25		13.33	0.83		5
Foxglove †	0.83			0		
Deschampsia caespitosa	0.83			1.25		
Heath Bedstraw †	0.83			0.83		
Ground ivy †	0.83			0		
Holly	0.83			2.08		
Hawthorn	0.83		6.67	0		
Molinia caerula	0.83			0		
Tormentil †	0.83		13.33	0		
Dactylis glomerata	0.42	0.08	13.33	0.83	2.78	75
Brachypodium sylvaticum	0.42		33.33	0		10
Bristly ox-tongue	0.42			0		
Honeysuckle	0.42			1.67		
Larch	0.42		46.67	0		0

Calluna vulgaris	0.42	-		0	-	
Selfheal †	0.42			0		
Nettle	0.42			0		
Carex spp	0.42	1.39	73.33	0.83	0.08	15
Dead leaves	0			2.08		
Daisy †	0			0.83		

Table A5.4 Squabb

Vegetation	Summer			Winter		
	Proportion available	**Proportion eaten**	**Proportion occur**	**Proportion available**	**Proportion eaten**	**Proportion occur**
Ivy	25.79	3.81	87.5	38.49	12.16	89.47
Hazel	11.51	0.05	12.5	0	0	0
Bracken	9.52	2.58	68.75	4.76	3.26	73.68
Honeysuckle	7.94		31.58	8.73		-
Bramble	7.94	33.02	100	9.92	55.33	100
Douglas fir	5.95		18.75	7.14		-
Moss (other)	4.37			7.14		
Herb	4.37	2.47	56.25	2.38	0	0
Silver birch	3.97	5.56	62.5	0	0.24	5.26
Beech	2.38	0.48	37.5			
Rape	0		38.84	2.38		0
Juncus spp.	1.98	6.4	50	2.78	0.04	5.26
Western hemlock	1.59	0.82	43.75	2.78	0.19	21.05
Holly	1.59			3.18		
Sycamore	1.59			0		
Holcus lanatus	1.19	0.08	12.5	1.59	0.04	10.53
Polytrichum spp.	1.19	0.81	50	2.38	0.52	52.63
Ground ivy †	1.19			0		
Foxglove †	1.19			0		

Germander speedwell †	0.79			0		
Hawthorn	0.79			0		
Rhododendron	0.79			0.79		
Elm	0.4			0		
Agrostis tenuis	0.4			1.19		
Oak	0.4	7.76	75	0	1.49	63.16
Carex spp.	0.4	2.66	93.75	0.79	3.26	100
Blackthorn	0.4			0		
Selfheal †	0.4			0.4		
Ash	0.4		12.5			
Spindle	0.4			0		
Agrostis canina	0.4			0.79		
Dactylis glomerata	0	0.29	18.75	0.4	0.45	21.05
Broom	0			0.4		
Dead leaves	0			5.95		
Common speedwell †	0			0.4		

APPENDIX 6

Nitrogen occurs in plants as nitrates (NO_3^-) and ammonia (NH_4^+) and it is in this form that herbivores take in nitrogen through eating the plants. Nitrogen is then incorporated into potentially large molecules such as proteins, nucleic acids and porphyrins, all critical to the survival of the animal. Proteins themselves constitute a very large group and include enzymes.

Nitrogen content can be converted into protein content by multiplying the former by a factor of 6.25, this is a unilaterally agreed constant (a calculated mean). By using this conversion factor two assumptions are made: firstly that all nitrogen in plants is present as protein and secondly that all plant protein contains 160g nitrogen per kg (McDonald *et al*, 1981). These assumptions have been made by many researchers and will also be accepted for this study.

Calcium exists as calcium cations (Ca^{2+}) and as such is absorbed from the soil into the plant which in turn is eaten. Calcium is involved in the selective permeability of plasma membranes and intracellular communication, activation of certain enzymes and the coagulation of blood. However its main function is structural as a component of bones, teeth and plant cell walls.

Magnesium occurs as magnesium cations (Mg^{2+}) and enters plants in this form and then herbivores. In plants it is a component of chlorophyll. Most (70%) of the total magnesium in an animals body is in the skeleton and the remainder is in the soft tissues and fluids. It is the commonest enzyme activator particularly in the phosphorylation process.

Potassium exists as potassium cations (K^+) in the soils and is taken up by plants in this form. In plants it is involved in the stomatal opening processes by maintaining the water status and turgor pressure of its cells. In animals it is important in the osmotic regulation of body fluids and in the anion-cation balance in cells. It also has an important role in nerve and muscle excitability and also activates enzymes.

Phosphorus occurs in an inorganic and organic form, dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^+), the two anionic forms are dependent on the soil pH and are taken into the plant. Phosphorus is a constituent of plasma membranes (phospholipids), certain protein (including enzymes), all nucleic acids and nucleotides. It is required for phosphorylation and is also found in bones and teeth.

Relationships between many of these nutrients exist, but the one mentioned most often is the Ca:P ratio. This ratio is referred to in nutritional studies and used in the manufacturing of animal feeds. It ranges from 1:1 to 2:1, although evidence suggests that ruminants can tolerate higher ratios (McDonald *et al*, 1981).

APPENDIX 7

Vegetation used for chemical analyses. Samples were initially chosen for analysis based upon their importance in the diet as established from the faecal analysis; plants comprising ≥1% of diet in any one bimonth. Others plants comprising <1% of the diet were selected that would be necessary to form composite groups, *e.g.* Herbs and also to allow inter-site comparisons.

Legend:

■ plant sample collected and ≥ 1% of diet in any one bimonth

□ plant sample collected but < 1% of the diet in all bimonths

Plant sample	**Brinken**	**Denny Lodge**	**Great Covert**	**Squabb**
Pine		■	■	■
Western hemlock		■	■	■
European larch		□	□	■
Douglas fir		□	■	□
Holly	■	■	□	□
Birch	■	■	■	■
Beech	□	■	□	■
Oak	■	■	■	■
Hazel			□	■
Gorse	□		□	■
Dwarf gorse	■			
Broom			□	□
Ivy	■	■	■	■
Bramble	■	□	■	■
Honeysuckle	■	■	□	□
Bracken	■	■	■	■
Calluna vulgaris	■		■	
Ragwort			■	
Polytrichum spp.	■	■	■	■

Holcus lanatus	☐	☐	☐	☐
Deschampsia caespitosa		■	☐	☐
Agrostis tenuis	☐	☐	☐	☐
A. canina	☐	☐	☐	☐
A. setacea		■		
Dactylis glomerata	■	■	■	■
Molinia caerula	■	☐	☐	
Sieglingia decumbens	■	■		
Brachypodium sylvaticum		☐	☐	
Juncus spp.	■	■	☐	■
Carex spp.	■	■	■	■
Wheat				■
White clover			■	☐
Galium spp.	☐	☐	☐	☐
Wood sorrel	☐	☐		☐
Ranunculus spp.		☐	☐	☐
Dandelion	☐	☐	☐	☐
Viola spp.	☐	☐	☐	☐
Tormentil	☐	☐	☐	☐
Wood sage		☐	☐	☐

APPENDIX 8

Legend:

shaded cells	similarities between the two studies
-	nutrient content of particular species not reported by Ekins (1989)

Table A8.1 Comparison between chemical analyses of commonly eaten forage by Ekins (1989) and in the current study.

Species	**Nitrogen**		**Calcium**		**Magnesium**		**Potassium**		**Phosphorus**	
	Current study	**Ekins (1989)**	**Current study**	**Ekins (1989)**	**Current study**	**Ekins (1989)**	**Current study**	**Ekins (1989)**	**Current study**	**Ekins (1989)**
Holly	low amounts / no difference over the year	late growing season, peak in June	peak JanFeb / lowest amounts MayJun	increase autumn / winter	peak MarApr and again in JulAug	content above average, increase autumn / winter	no difference over year	late growing season, peak in Jun	no difference over year	late growing season, peak Jun
Bramble	peak MayJun	peak Jun	peak NovDec, lowest MayJun	highest values of any other forage, peak in Mar and Nov	peak in JulAug, high values all year round	highest values of any other forage, peak Mar and Sep	peak in MayJun	peak May lowest Jan, Feb and Mar	peak JulAug	peak May / Jun, lowest Mar
Ivy	low amounts / no difference over year	-	peak JanFeb / MarApr, lowest amounts MayJun	-	peak SepOct	-	high throughout year, peak JulAug	-	peak JulAug	-
***Polytrichum* spp.**	low amount very little variation, lowest SepOct	-	very low amounts / no difference over year	low amounts	very low amounts	low amounts	low amounts but peak JulAug	low amounts	very low amounts	-
Oak	peak MayJun	peak May	peak NovDec	peak Sep, Oct and Jan	peak in JulAug	peak in May	peaks in MayJun and SepOct	peak May	peak MayJun	peak May
Birch	peak MarApr	-	peak SepOct / NovDec	-	peak JulAug	-	peak MarApr	-	peak MarApr	-

Table A8.1 *continued*

Species	Nitrogen		Calcium		Magnesium		Potassium		Phosphorus	
	Current study	Ekins (1989)	Current study	Ekins (1989)	Current study	Ekins (1989)	Current study	Ekins (1989)	Current study	Ekins (1989)
***Carex* spp.**	peak MayJun	-	very low amounts, lowest SepOct	-	low amoutns	-	high amounts peak JulAug	-		-
***Juncus* spp.**	peak MayJun	peak Apr / May	very low amounts peak SepOct	very low amounts, small peak Sep	low amounts	steady increase peak in Oct	high amounts MayJun / JulAug peaks	high amounts peaks over Apr, May Jun	peak MayJun	peak over Apr May Jun
Bracken	peak MayJun	high levels Apr	peak MarApr large decrease MayJun / JulAug	very low amounts in Apr	peak in NovDec / JanFeb	high amounts all year	peak MayJun / JulAug, very low JanFeb/ MarApr	high levels April	peak MayJun	high levels Apr
Pine	low amounts, no difference over year	low amounts	peak NovDec / JanFeb	low amounts	peak in MayJun	low amounts	low amounts peak SepOct	low amounts	peak MayJun and SepOct	low amounts
Douglas fir	low amounts peak MayJun	-	peak MarApr no real difference over year	-	peak in MarApr	-	very low amounts	-	low amounts peak MayJun	-
Western hemlock	low amounts peak JanFeb, lowest SepOct	-	peak JanFeb	-	no difference over year	-	very low amoutns	-	peaks in MarApr	-

APPENDIX 9

Table A9.1 Nitrogen content (% dry weight) of vegetation through the seasons, published values and data from each site (values in italics indicate converted from published protein values where crude protein = nitrogen content x 6.25)

	spring	**summer**	**autumn**	**winter**
Hellmers (1940) (range)				*0.67 - 1.46*
Swift (1948) (mean)		*3.16*		
Smith (1957) (mean)				*1.18*
Dietz *et al* (1958) (means)	*1.88*	*1.60*	*1.36*	*1.54*
Short *et al* (1966) (range)	*1.28 - 2.72*		*0.96 - 1.6*	*0.96 - 1.12*
Torgerson and Pfander (1971) (means)		*2.4*		*0.85*
Radwan and Crouch (1974) (mean)				1.17
Ekins (1989) (range of means)		0.9 - 2.2 (May-Oct)		0.79 - 1.44 (Nov-Apr)
Brinken (means)	2.45	1.92	1.81	2.10
Denny Lodge (means)	2.72	2.22	1.93	1.67
Great Covert (means)	2.48	2.24	2.07	1.87
Squabb (means)	2.34	2.24	2.05	2.00

Table A9.2 Calcium content (% dry weight) of vegetation through the seasons, published values and data from each site.

	spring	summer	autumn	winter
Swift (1948) (mean)				0.953
Dietz *et al* (1958) (means)	1.04	1.28	1.03	0.93
Short *et al* (1966) (range)			1.14 - 1.68	
Torgerson and Pfander (1971) (means)		1.95		0.82
Radwan and Crouch (1974) (mean)				1.2
Ekins (1989) (range of means)		0.10 - 0.7 (May-Oct)		0.07 - 1.04 (Nov-Apr)
Brinken (means)	0.28	0.28	0.32	0.19
Denny Lodge (means)	0.29	0.29	0.35	0.36
Great Covert (means)	0.38	0.41	0.42	0.34
Squabb (means)	0.35	0.35	0.45	0.40

Table A9.3 Magnesium content (% dry weight) of vegetation through the seasons, published values and data from each site.

	spring	summer	autumn	winter
Swift (1948) (mean)				0.235
Short *et al* (1966) (range)		0.25 - 0.28		
Radwan and Crouch (1974) (mean)				0.208
Ekins (1989) (range of means)		0.09 - 0.29 (May-Oct)		0.02 - 0.3 (Nov-Apr)
Brinken (means)	0.19	0.14	0.16	0.14
Denny Lodge (means)	0.19	0.17	0.17	0.17
Great Covert (means)	0.17	0.19	0.17	0.13
Squabb (means)	0.18	0.24	0.21	0.17

Table A9.4 Potassium content (% dry weight) of vegetation through the seasons, published values and data from each site.

	spring	summer	autumn	winter
Swift (1948) (mean)				2.62
Short ***et al*** **(1966)** (range)		0.5 - 3.1	1.8 max	0.32 - 0.92
Torgerson and Pfander (1971) (means)		2.41		0.79
Ekins (1989) (range of means)		1.02 - 2.9 (May-Oct)		0.09 -1.03 (Nov-Apr)
Brinken (means)	1.52	1.07	1.5	1.27
Denny Lodge (means)	2.0	2.13	1.68	1.13
Great Covert (means)	2.21	2.56	2.15	1.50
Squabb (means)	2.46	2.75	2.21	1.68

Table A9.5 Phosphorus content (% dry weight) of vegetation through the seasons, published values and data from each site.

	spring	summer	autumn	winter
Swift (1948) (mean)				0.40
Dietz (1958) ***et al*** (means)	0.25	0.21	0.17	0.19
Short ***et al*** **(1966)** (range)	0.21 - 0.49	0.15 - 0.36	0.2 - 0.49	0.09 - 0.18
Torgerson and Pfander (1971) (means)		0.25		0.11
Radwan and Crouch (1974) (mean)				0.18
Ekins (1989) (range of means)		0.11 - 0.32 (May-Oct)		0.04 - 0.15 (Nov-Apr)
Brinken (means)	0.12	0.07	0.09	0.11
Denny Lodge (means)	0.15	0.09	0.09	0.10
Great Covert (means)	0.10	0.09	0.09	0.06
Squabb (means)	0.10	0.11	0.09	0.10

APPENDIX 10

Table A10.1 Protein content of the three most commonly eaten plant species in each bimonth at all sites.

Bimonth	**Brinken**		**Denny Lodge**		**Great Covert**		**Squabb**	
	Common plant species eaten	***Protein content (%CP)***	***Common plant species eaten***	***Protein content (%CP)***	***Common plant species eaten***	***Protein content (%CP)***	***Common plant species eaten***	***Protein content (%CP)***
JanFeb	Ivy	10.4	Moss	11.8	Douglas fir	11.2	Pine	12.2
	Moss	10.2	Pine	9.7	Ivy	10.9	Bramble	10.7
	Holly	6.8	Holly	7.9	Western hemlock	8.7	Ivy	9.0
MarApr	Moss	11.7	Monocotyledon	20.3	Monocotyledon	17.6	Ivy	13.5
	Holly	10.8	Moss	11.6	Douglas fir	9.5	Pine	9.5
	Ivy	7.9	Pine	11.2	Western hemlock	7.5	Bramble	8.1
MayJun	Monocotyledon	17.0	Monocotyledon	18.6	Oak	26.8	Birch	19.5
	Oak	12.6	Moss	11.1	Monocotyledon	16.1	Oak	13.6
	Moss	8.8	Coarse grass	16.3	Western hemlock	6.3	Bramble	11.6
JulAug	Oak	13.5	Monocotyledon	15.0	Bramble	23.2	Oak	16.7
	Bramble	12.2	Coarse grass	14.9	Deciduous tree	19.3	Ivy	11.5
	Moss	8.2	Moss	9.8	Western hemlock	10.0	Bramble	9.8
SepOct	Bramble	13.1	Deciduous tree	11.6	Deciduous tree	17.9	Oak	19.5
	Moss	8.1	Moss	11.1	Bramble	13.7	Bramble	14.3
	Holly	6.1	Pine	9.8	Western hemlock	6.6	Ivy	7.4
NovDec	Bramble	14.7	Moss	12.5	Oak	17.3	Ivy	10.9
	Moss	9.6	Pine	11.4	Western hemlock	10.4	Bramble	12.3
	Holly	7.8	Deciduous tree	9.7	Douglas fir	8.0	Deciduous tree	10.1

Table A10.2 Calcium content of the three most commonly eaten plant species in each bimonth at all sites.

Bimonth	Brinken		Denny Lodge		Great Covert		Squabb	
	Common plant species eaten	*Calcium content (%)*	*Common plant species eaten*	*Calcium content (%)*	*Common plant species eaten*	*Calcium content (%)*	*Common plant species eaten*	*Calcium content (%)*
JanFeb	Ivy	0.66	Holly	0.75	Ivy	0.76	Ivy	1.0
	Holly	0.56	Pine	0.54	Douglas fir	0.58	Bramble	0.7
	Moss	0.08	Moss	0.10	Western hemlock	0.47	Pine	0.43
MarApr	Holly	0.54	Pine	0.19	Douglas fir	0.81	Ivy	1.13
	Ivy	0.42	Monocotyledon	0.16	Western hemlock	0.22	Bramble	0.9
	Moss	0.12	Moss	0.06	Monocotyledon	0.15	Pine	0.52
MayJun	Oak	0.45	Monocotyledon	0.15	Western hemlock	0.43	Birch	0.32
	Monocotyledon	0.12	Moss	0.08	Oak	0.23	Oak	0.3
	Moss	0.05	Coarse grass	0.06	Monocotyledon	0.15	Bramble	0.23
JulAug	Oak	0.30	Monocotyledon	0.13	Deciduous tree	0.46	Ivy	0.87
	Bramble	0.23	Coarse grass	0.12	Bramble	0.20	Bramble	0.56
	Moss	0.05	Moss	0.08	Western	0.16	Oak	0.42
SepOct	Holly	0.41	Deciduous tree	0.54	Deciduous tree	0.58	Ivy	1.09
	Bramble	0.35	Pine	0.35	Bramble	0.48	Oak	0.87
	Moss	0.04	Moss	0.11	Western hemlock	0.19	Bramble	0.71
NovDec	Holly	0.48	Deciduous tree	0.64	Oak	0.86	Bramble	0.95
	Bramble	0.39	Pine	0.52	Western hemlock	0.26	Ivy	0.87
	Moss	0.07	Moss	0.11	Douglas fir	0.18	Deciduous tree	0.39

Table A10.3 Magnesium content of the three most commonly eaten plant species in each bimonth at all sites.

Bimonth	**Brinken**		**Denny Lodge**		**Great Covert**		**Squabb**	
	Common plant species eaten	***Magnesium content (%)***	***Common plant species eaten***	***Magnesium content (%)***	***Common plant species eaten***	***Magnesium content (%)***	***Common plant species eaten***	***Magnesium content (%)***
JanFeb	Ivy	0.18	Pine	0.13	Ivy	0.21	Ivy	0.25
	Moss	0.15	Moss	0.12	Douglas fir	0.12	Bramble	0.25
	Holly	0.05	Holly	0.12	Western hemlock	0.09	Pine	0.10
MarApr	Moss	0.19	Monocotyledon	0.18	Monocotyledon	0.14	Bramble	0.33
	Holly	0.11	Moss	0.09	Douglas fir	0.13	Pine	0.09
	Ivy	0.08	Pine	0.09	Western hemlock	0.07	Ivy	0.09
MayJun	Monocotyledon	0.20	Monocotyledon	0.18	Oak	0.19	Oak	0.18
	Moss	0.11	Coarse grass	0.10	Monocotyledon	0.16	Bramble	0.18
	Oak	0.09	Moss	0.1	Western hemlock	0.10	Birch	0.12
JulAug	Bramble	0.23	Coarse grass	0.15	Bramble	0.36	Bramble	0.54
	Moss	0.09	Monocotyledon	0.14	Monocotyledon	0.18	Oak	0.35
	Oak	0.09	Moss	0.08	Western	0.08	Ivy	0.18
SepOct	Bramble	0.19	Pine	0.11	Bramble	0.20	Bramble	0.52
	Holly	0.09	Deciduous tree	0.1	Deciduous tree	0.15	Ivy	0.22
	Moss	0.08	Moss	0.09	Western hemlock	0.07	Oak	0.14
NovDec	Bramble	0.21	Pine	0.23	Oak	0.24	Bramble	0.31
	Moss	0.11	Deciduous tree	0.12	Western hemlock	0.12	Ivy	0.17
	Holly	0.09	Moss	0.1	Douglas fir	0.09	Deciduous tree	0.07

Table A10.4 Potassium content of the three most commonly eaten plant species in each bimonth at all sites.

Bimonth	**Brinken**		**Denny Lodge**		**Great Covert**		**Squabb**	
	Common plant species eaten	***Potassium content (%)***	***Common plant species eaten***	***Potassium content (%)***	***Common plant species eaten***	***Potassium content (%)***	***Common plant species eaten***	***Potassium content (%)***
JanFeb	Ivy	0.8	Holly	1.15	Ivy	2.31	Ivy	2.64
	Moss	0.51	Moss	0.83	Western hemlock	0.82	Pine	0.86
	Holly	0.32	Pine	0.49	Douglas fir	0.61	Bramble	0.52
MarApr	Ivy	0.91	Monocotyledon	3.19	Monocotyledon	2.60	Ivy	2.61
	Moss	0.44	Moss	0.71	Ivy	1.83	Pine	0.86
	Holly	0.40	Pine	0.66	Western hemlock	0.49	Bramble	0.83
MayJun	Monocotyledon	2.5	Monocotyledon	3.73	Monocotyledon	3.43	Bramble	2.1
	Oak	1.16	Coarse grass	2.3	Oak	2.09	Birch	1.9
	Moss	0.31	Moss	0.6	Western hemlock	0.55	Oak	1.19
JulAug	Oak	0.87	Coarse grass	2.99	Bramble	1.9	Ivy	2.88
	Bramble	0.73	Monocotyledon	2.75	Deciduous tree	1.38	Bramble	1.37
	Moss	0.44	Moss	1.07	Western	1.21	Oak	0.78
SepOct	Bramble	0.62	Deciduous tree	1.06	Bramble	1.35	Ivy	2.55
	Holly	0.41	Moss	0.54	Deciduous tree	1.17	Oak	1.16
	Moss	0.35	Pine	0.44	Western hemlock	0.7	Bramble	1.12
NovDec	Bramble	0.46	Deciduous tree	2.61	Douglas fir	1.06	Deciduous tree	0.94
	Moss	0.4	Moss	0.64	Western hemlock	0.91	Ivy	2.38
	Holly	0.34	Pine	0.52	Oak	0.84	Bramble	1.22

Table A10.5 Phosphorus content of the three most commonly eaten plant species in each bimonth at all sites.

Bimonth	**Brinken**		**Denny Lodge**		**Great Covert**		**Squabb**	
	Common plant species eaten	***Phosphorus content (%)***	***Common plant species eaten***	***Phosphorus content (%)***	***Common plant species eaten***	***Phosphorus content (%)***	***Common plant species eaten***	***Phosphorus content (%)***
JanFeb	Ivy	0.11	Holly	0.14	Douglas fir	0.07	Pine	0.14
	Moss	0.05	Pine	0.09	Ivy	0.05	Ivy	0.11
	Holly	0.04	Moss	0.07	Western hemlock	0.04	Bramble	0.03
MarApr	Moss	0.06	Monocotyledon	0.26	Monocotyledon	0.19	Ivy	0.13
	Holly	0.05	Pine	0.1	Western hemlock	0.08	Pine	0.13
	Ivy	0.05	Moss	0.01	Ivy	0.06	Bramble	0.09
MayJun	Oak	0.13	Monocotyledon	0.17	Oak	0.13	Bramble	0.17
	Monocotyledon	0.17	Coarse grass	0.09	Monocotyledon	0.12	Birch	0.13
	Moss	0.04	Moss	0.01	Western hemlock	0.06	Oak	0.04
JulAug	Bramble	0.12	Monocotyledon	0.1	Bramble	0.35	Oak	0.16
	Oak	0.07	Coarse grass	0.1	Western	0.1	Ivy	0.13
	Moss	0.05	Moss	0.01	Deciduous tree	0.07	Bramble	0.13
SepOct	Bramble	0.05	Pine	0.08	Deciduous tree	0.09	Oak	0.16
	Holly	0.04	Deciduous tree	0.08	Western hemlock	0.09	Bramble	0.12
	Moss	0.04	Moss	0.02	Bramble	0.08	Ivy	0.11
NovDec	Bramble	0.08	Deciduous tree	0.13	Western hemlock	0.09	Deciduous tree	0.1
	Moss	0.07	Pine	0.07	Douglas fir	0.07	Ivy	0.1
	Holly	0.06	Moss	0.02	Oak	0.07	Bramble	0.03

APPENDIX 11

Recipes for the diets

Tables - Recipes for the respective diets as used in Trials 1-3.

Legend:

Wgt = weight in grammes (g)

DM = dry matter in grammes per kilogramme (g/kg)

CP = crude protein as in grammes dry matter or as a percentage (%) as fed

ME = metabolisable energy in megajoules per kilogramme dry matter (MJ/kg DM) or kilojoules per gramme dry matter (KJ/g DM)

All calculations were made using published values in terms of dry matter, this is a standard procedure when using agricultural food materials. The shaded box in each table shows the final crude protein content (%) on a dry matter basis.

Calculations:

DM calculated = multiplication of published DM (converted to a proportion on a 0-1 scale, *i.e.* divided by 1000) and actual weight of ingredient used.

CP calculated = multiplication of published percentage (converted to a proportion on a 0-1 scale, *i.e.* divided by 100) and calculated DM.

ME calculated = multiplication of published ME values and calculated DM

CP % DM = division of total CP calculated by total DM calculated multiplied by 100, giving a percentage.

ME MJ/kg DM = division of total ME (KJ/g DM) calculated by total DM calculated.

Table A11.1 TRIAL 1: 9.35% Protein diet 'low protein' (LP1)

		published values*			calculated values		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g DM)**	**ME (MJ DM)**
Hay	79	850	8.5	8.4	67.15	5.71	564.06
Molasses	15	773	13.6	11.8	11.6	1.58	136.82
Flaked maize	5	889	11	14	4.45	0.49	62.23
Vit/min suppl.	1						
total	100				83.2	7.78	763.11
%CP DM						**9.35**	
ME KJ DM							9.17

Table A11.2 TRIAL 1: 15.71% protein diet 'high protein' (HP1)

		published values*			calculated values		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g)**	**ME (MJ DM)**
Hay	80	850	8.5	8.4	68	5.78	571.2
Molasses	2	773	13.6	11.8	1.55	0.21	18.24
Flaked maize	2	889	11	14	1.78	0.2	24.89
Soya bean meal	15	887	53.4	13.7	13.31	7.11	182.28
Vit/min suppl.	1						
total	100				84.64	13.3	796.61
%CP DM						**15.71**	
ME (MJ/kg) DM							9.41

Table A11.3 TRIAL 2: 13.65% protein diet 'optimum protein' (OP2)

		published values*			calculated values		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g)**	**ME (MJ DM)**
Barley	91.3	875	12.1	13.3	79.89	9.67	1062.54
Fish meal	2.7	900	70.1	11.1	2.43	1.70	26.97
Vit/min suppl.	1						
total	100				83.32	11.37	1089.51
%CP DM						**13.65**	
ME (MJ/kg) DM							13.24

Table A11.4 TRIAL 2: 26.88% protein diet 'high protein' (HP2)

		published values*			calculated values		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g)**	**ME (MJ DM)**
Barley	67.3	875	12.1	13.3	58.88	7.12	783.1
Fish meal	11.6	900	70.1	11.1	10.44	7.32	115.88
Molasses	4	773	13.6	11.8	3.09	0.41	36.46
Soya bean meal	15.6	887	53.4	13.7	13.84	8.33	189.61
Vit/min suppl.	1.5						
total	100				86.25	23.18	1125.05
%CP DM						**26.88**	
ME (MJ/kg) DM							13.04

Table A11.5 TRIAL 3: 13.54% protein diet ‘optimum protein’ (OP3)

		published values*			**calculated values**		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g)**	**ME (MJ DM)**
Barley	33	875	12.1	13.3	28.88	3.49	384.04
Wheat	30	871	12.2	13.8	26.13	3.19	360.59
Molasses	2	773	13.6	11.8	1.55	0.21	18.24
Beet pulp	3	889	9.9	12.7	2.67	0.26	33.87
Wheat feed	28	888	17	10.5	24.86	4.23	261.07
Vit/min suppl.	4						
total	100				84.08	11.38	1057.82
%CP DM						**13.54**	
ME (MJ/kg) DM							12.58

Table A11.6 TRIAL 3: DUKE'S DEER CHOICE 18 NUTS - 21.89% protein diet 'high protein' (HP3)

		published values*			calculated values		
Ingredients	**Wgt (g)**	**DM (g/kg)**	**CP %**	**ME (MJ/kg DM)**	**DM (g/kg)**	**CP (g)**	**ME (MJ DM)**
Soya bean meal	4.184	887	53.4	13.7	3.71	1.98	50.84
Rape meal	8	915	35.4	11.4	7.32	2.59	83.45
Sunflower meal	17.5	893	34.8	9.4	15.63	5.44	146.9
Lucerne meal	2.778	918	16.5	8.7	2.55	0.42	22.19
Maize gluten	6.257	871	22.7	12.9	5.45	1.24	70.3
Molasses**	5	773	3.49	11.8	3.87	0.14	45.61
Beet pulp	16.55	889	9.3	12.7	14.71	1.37	186.85
Wheat feed	35	888	17	10.5	31.08	5.28	326.34
Fat310	1						30.96
Vit/min suppl.	3.731						
total	100				84.32	18.46	963.95
%CP DM						**21.89**	
ME (MJ/kg) DM							11.43

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* published values from BOCM PAULS (1996), van Soest (1994) and McDonald *et al* (1981).

** the data for molasses varies depending on its origin In the BOCM PAULS Deer Choice 18 Nuts the molasses originated from sugar cane, which is lower in protein content, whereas all the other diets contained molasses from sugar beet.

APPENDIX 12

Table A12.1 Length of time deer ate for on each day of Trial One (figures in bold indicate days where deer fed for less than 30 minutes, the cut off point for calculating the preference scores).

Day	Paultons Park (minutes)	Magdalen College (minutes)	Stonor (minutes)
1	**16**	60	**24**
2	30	60	**13**
3	36	40	-
4	45	**26**	-
5	51	35	30
6	70	31	-
7	**29**	36	-
8	62	**22**	-
9	75	33	-
10	30	30	30
11	60	-	30

Table A12.2 Length of time deer ate for on each day of Trial Three (figures in bold indicate days where deer fed for less than 16 minutes, the cut off point for calculating the preference scores).

Day	Paultons Park (minutes)	Magdalen College (minutes)	Stonor (minutes)	Nature Quest (minutes)	White House Farm (minutes)
1	30	16	19	20	**7**
2	16	30	26	28	16
3	30	25	33	24	25
4	21	22	27	24	-
5	28	25	44	21	16
6	16	17	40	26	20
7	18	21		24	16
8	16	18		16	18
9	19			16	20
10	16				16

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