INFLUENCE OF THE ENDOPHYTE FUNGUS IN RYEGRASS ON GRAZING PREFERENCE BY SHEEP

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Animal productivity may be enhanced through greater intake associated with the use of preferred pasture species provided they are of high nutritional value. Grazing preference by ewe hoggets was investigated in three grazing trials and an indoor feeding trial.

The first grazing trial (April 3-12, 1990) examined the preference of ewe hoggets for three grass treatments, each containing white clover. The grass treatments were endophyte (Acremonium lolii) infected (+E) perennial ryegrass, endophyte free (-E) perennial ryegrass and tall fescue. Two 0.2 ha blocks, each containing three replicates of the three grass treatments were sown in spring 1989. Endophyte incidence (% infected tillers) was 40% in +E ryegrass and 0% in -E ryegrass. Tillers were vegetative. Ewe hoggets (15) were introduced into the plots and their preferences recorded by visual observations from a raised viewing tower, and agronomic measurements on the plots. On day 1 of grazing a high proportion (76%) of ewe hoggets grazed tall fescue while a low proportion (12%) grazed both +E and -E ryegrass. From day 2 to 10 of grazing a similar number of sheep grazed each grass treatment. Grass leaf height declined less in +E ryegrass (48%), than -E ryegrass (64%) and tall fescue (71%) during grazing. Pseudostem height of grasses was reduced 4.2mm in tall fescue and -E ryegrass but only 1.5mm in +E ryegrass by grazing. Pasture mass declined more in -E ryegrass (1230 kg DM/ha) than +E ryegrass (1100 kg DM/ha) or tall fescue (1020 kg DM/ha). The percentage green grass cover (point analysis first hits) decreased with grazing in all grass treatments but less in +E ryegrass (21%) than -E ryegrass (34%) and tall fescue (28%). White clover percentage was low in all grass treatments (4-7%) prior to grazing and declined with grazing. Both +E and -E ryegrass contained more dead material than tall fescue.

In a second grazing trial (May 14-21) the same plots were either mown to 30mm or left unmown two weeks prior to grazing and also subdivided into urine and non-urine patches. No endophyte was detected in either +E or -E ryegrass. Ewe hoggets (20) were introduced and agronomic measurements made during grazing. The height of grass leaves in all treatments was always greater in urine than non-urine patches but grass leaf height declined more rapidly in urine patches. The grass leaf height of +E and -E ryegrass was twice that of tall fescue in urine and non-urine patches throughout grazing. Pasture mass, prior to grazing, over all treatments was higher (1990 vs 1578 kg DM/ha) but declined more during grazing (928 v 751 kg DM/ha) in urine than non-urine patches. Mowing created a constant pseudostem height (30-32 mm) but did not influence pasture mass or grass leaf height changes.

In the third grazing trial (25 September - 5 October) measurements of grass leaf height were made throughout grazing on +E (60% infection) ryegrass and -E ryegrass (0% infection) plots that were part of a grazing preference study consisting of four replicates of eight grasses or herbs. Plots had been ungrazed since May 23, 1990. Reproductive tiller development had begun. The height of +E and -E ryegrass were similar prior to grazing (180mm) but declined more in -E ryegrass (135mm) than +E ryegrass (80mm). Endophyte incidence in laxly grazed areas of +E ryegrass plots was 70% but only 30% in intensively grazed areas.

The rate of eating of different feeds (white clover, tall fescue, +E ryegrass (70% infection) and -E ryegrass (0% infection)) was measured by offering 100g fresh samples of each alone to eight hungry sheep and recording time taken to consume each sample. Eating rates were highest for white clover (5.52 g DM/min) and lowest for +E ryegrass (2.89 g DM/min) with tall fescue (3.86 g DM/min) and -E ryegrass (3.94 g DM/min) intermediate. The three grass feeds were then separated into pseudostem and leaf, and the rate of eating measured. Leaf material was eaten at similar rate for all grasses (3.86-4.21 g DM/min). The +E ryegrass pseudostems were eaten more slowly (1.85 g DM/min) than -E ryegrass (2.65 g DM/min) or tall fescue (3.47 g DM/min).

The slower rate of decline of grass leaf height, the smaller amount of pseudostem, pasture mass and green leaf removed, and the lower rate of eating of endophyte infected +E ryegrass when compared to -E ryegrass is interpreted as discrimination by sheep against endophyte infected +E ryegrass. It is proposed that low animal production of sheep grazing +E ryegrass can be attributed to the reduced intake of +E ryegrass because of avoidance behaviour. It is also suggested that the avoidance of sheep by fungus from eating the grass, is an adaptive mechanism of the fungus to ensure continued survival via seed spread.

Keywords

Acremonium lolii, Lolium perenne, Festuca arundinacea, Trifolium repens, perennial ryegrass, white clover, tall fescue, lolium endophyte, grazing preference, eating rate, avoidance.

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CHAPTER 1
INTRODUCTION

New Zealand pastures are based typically on grass/legume associations. Perennial ryegrass (Lolium perenne L.) is still the major grass species although the use of alternative species is increasing. Perennial ryegrass is preferred by farmers for its relative persistence, productivity and fast establishment qualities on many soil types and in many climates and this is reflected in research investment (Hunt and Easton, 1989). The last decade has seen the initiation of renewed interest and research into perennial ryegrass. This work has mostly been associated with the lolium endophyte dilemma.

The presence of the fungal hyphae of the perennial ryegrass endophyte (Acremonium lolii) growing inside ryegrass leaf tissue has been known for many years (Neill, 1940). However, the agricultural implications of this endophyte are only beginning to be realised. Research into the relationship between A. lolii, perennial ryegrass and animal production has exposed several contradictory consequences of endophyte presence. The presence of endophyte has been linked to the neuromuscular disorder ryegrass staggers, low liveweight gains in sheep during spring and reduced serum prolaction levels in sheep (Fletcher and Harvey, 1981; Fletcher, 1983; Fletcher and Barrell, 1984). Sutherland and Hoglund (1989) found high endophyte ryegrass to suppress clover growth and seedlings survival to the extent that soil nitrogen levels were lowered sufficiently to reduce subsequent crop yields.

Conversely, improved ryegrass persistence and production during and after summer drought has also been linked to endophyte presence owing to reduced Argentine stem weevil damage (Listronotus bonariensis) (Mortimer et. al., 1982, Gaynor and Hunt, 1983). In addition, under optimum plant growth conditions A. lolii has been shown to increase plant growth (Latch et. al., 1985).

There are now some 64 million sheep and 8 million cattle in New Zealand (NZ Official Year Book, 1990) grazing for most of the year on pasture of mixed botanical composition. However, in New Zealand, a country which has achieved fame for its enlightened advances in pasture science, it is surprising that only recently research has been conducted into the dietry preferences of domestic animals for particular plant species - information fundamental to any assessment of the value of mixed swards in animal nutrition.

Euphagic behaviour, or the selection of dietry components, is the dominant response of herbivores presented with a choice of potential forage. A difference between the diet harvested by animals and the composition of the total sward on offer is an indication of selection. Animals consistently select more leaf than stem and more green than dead material compared with the sward from which it is harvested (Van Dyne et. al., 1980; Geenty, 1983). The material eaten, when compared with material on offer, usually has a higher content of nitrogen, phosphate and gross energy, but is lower in fibre content (Arnold, 1981). Ease of prehension may be important in diet selection, leaf has a lower structural strength and sheer force than stem (Heady, 1975).

Selection in mixed swards may depend on plant species involved. While sheep appear to graze relatively indiscriminantly at the surface of intensively managed ryegrass white clover (Trifolium repens.L) swards (Milne et. al., 1982) there is evidence for selection of white clover from within all horizons in a fescue - white clover sward (Hodgson and Grant, 1980). Barthram and Grant (1984) have shown that sheep readily graze within the leafy horizon but are reluctant to penetrate into lower horizons containing pseudostem and dead material. Prairie grass was most preferred by horses, low oestrogen red clover by deer and cocksfoot by calves (Hunt and Hay, 1990).

Pasture species preferences by sheep have in the past been considered important to the full utilisation and stability of pasture (Ivins 1955) and have been judged influential on animal intake (Tribe and Gordon, 1950). It is inferred that animal productivity could be increased through the use of the most palatable pasture species, provided they are of high nutritional value.

The low live-weight gains of sheep grazing endophyte infected ryegrass (Fletcher, 1986), the decrease in voluntary intake of endophyte infected tall fescue (Goetsch et. al., 1987) and farmers observations that areas of high endophyte pasture are left ungrazed, prompted the investigations of this study. The objective was to determine whether the endophyte fungus A. lolii influences grazing preference in sheep.

The grazing preference of ewe hoggets for three grasses - tall fescue (Festuca arundinaces) plus (+E) endophyte ryegrass and minus (-E) endophyte ryegrass all associated with white clover was examined during two autumn grazing periods. Visual observations and agronomic measurements of pastures were used to assess preference. Agronomic measurements of pastures in spring were also made to assess preference for +E and -E ryegrass.

The effect of the different feed types available to the sheep in the grazing trial (above) on the short term rate of eating was studied in an indoor feeding trial.

CHAPTER 2
REVIEW OF LITERATURE

PART 1 : GRASS ENDOPHYTES

2.1 INTRODUCTION

The term endophyte (Greek : endo = within + phyte = plant) has been defined as an organism contained or growing (entirely) within the substrate plant, whether parasitically or not (Walker, 1950). If the term was used in its broadest sense, this review could include all fungi that spend all or nearly all of their life cycle in the host grasses. However, endophytic fungi such as smutts and vesicular - arbuscular mycorrhizas that infect grasses will not be discussed. Of interest to agricultural researchers is a specific group of clavicipitaceous fungi that belong or are related to fungi of the tribe Balansiae (Bacon et. al., 1986). It has long been recognised that these fungi exist in certain grasses such as Lolium (Neil, 1940). Such fungi are either true endophytes that never produce external fructifications on the plant or else may produce external mycelium and/or spores that affect flower and seed production (Siegel et. al., 1987). Endophytes of agricultural grasses have been extensively reviewed by Siegel et. al. (1987) and Pottinger et. al. (1985).

Initial work of Bacon et. al., (1977) and Fletcher and Harvey (1981) reported close associations of endophyte infected tall fescue (Festuca arundinacea) and perennial ryegrass (Lolium perenne) with the animal maladies fescue toxicosis and ryegrass staggers. Since these discoveries dramatic advances have been made in the understanding of the relationship between fungal endophytes of grasses and animal and/or pasture performance. This understanding includes the origin of and incidence of infected grasses modes of dissemination of fungi, identification of chemicals responsible for toxicoses and control of fungi. It has also become recognised that grass endophytes can play an important role in survival of host plants subjected to environmental stresses (insects, drought, grazing animals).

This chapter therefore reviews the beneficial and harmful effects of endophytic fungi on pasture grasses and grazing animals. It examines classification, incidence, plant-fungus relationships and fungus-animal relationships focusing specifically on those grass-endophyte associations relevant to New Zealand agriculture.

2.2 CLASSIFICATION OF ENDOPHYTES

In their review of fungal endophytes Siegel, et. al. (1987) reported that the endophytes of grasses and sedges were grouped in the tribe Balansiae of Clavicipitaceae. Within this tribe are the genera Balansiopsis, Atkinsonella, Myriogenospora, Balansia, and Epichloe (Luttrell & Bacon, 1977). However there is conflicting opinion over taxonomy of endophytes. The section Albo - lanosa in genus Acremonium was created by Morgan-Jones and Gams (1982) to accommodate the endophyte commonly found in tall fescue. They named this fungus Acremonium coenophialum, and subsequently, endophytes in other species of Festuca and Lolium have been placed in this section (Latch et. al., 1984; White et. al., 1987; White, Morgan-Jones and Gams, 1987). Rykard et. al. (1985) maintain that it would be preferable to leave the newly named Acremonium species in the genus Sphacelia, the conidial state of Epichloe typhina. Definite classification relies on finding the teleomorphic states of fungi.

Latch et al (1984) have described two seed born endophytes with Gliocladium-like and Phialophora-like conidia in perennial ryegrass and tall fescue respectively. The exact relationship of these endophytes to Balansiae is unknown.

All genera of the tribe Balansiae, except for Myriogeospora, produce systematic infections in leaves, culms and inflorescences, becoming conspicuous when sporulation occurs as the plant flowers. Some endophytes interfere with reproduction, whereas others have no influence (Siegel et. al., 1987). Intercellular hyphae have been found in the ovary wall and throughout nucellar tissue of perennial ryegrass ovules (Lloyd, 1959; Philipson & Christey, 1986). Additionally, at megametophyte maturity in perennial ryegrass, hyphae of

Acremonium lolii become intracellular by penetrating the embryo sac soon after fertilization (Philipson & Christey, 1986). With embryo enlargement differentiation occurs and hyphae pass into the shoot apical region. Therefore, some endophytes are efficiently dispersed with their host seed and have no need for spores as means of dissemination. It is believed that the only means of dissemination of Acremonium endophytes is through maternal transmission of infected seed (Latch, 1983).

2.3 DESCRIPTION OF MYCELIUM IN PLANTS

Mycelia of A. lolii, A. coenophialum, and the Acremonium-like anamorph of Epichloe typhina have septate intercellular infrequently branched hyphae running parallel to the leaf axis. A. coenophialum differs from the other two in that the mycelium are slightly more convulted (Latch et. al., 1984).

Mycelia of the Gliocladium-like endophyte is strongly branched but often make identification of A. lolii difficult when both occur together (Latch et. al., 1984).

2.4 INCIDENCE

2.4.1 INCIDENCE OF ENDOPHYTES IN GRASSES

Siegel et. al., (1987) reviewed the incidence of endophyte in grass plants reporting species of Balansia, Balansiopsis, Atkinsonella, and Myriogenospora infect a wide range of tropical C4 plants. Most of these grasses generally are regarded as weeds and have minor agricultural importance (Luttrell and Bacon, 1977). Epichloe typhina is found on genera of forage and turf grasses of Agrostis, Dactylis, Festuca, Holcus, Hordeum, and Lolium (Siegel et. al., 1987). Endophytes that have Acremonium, Sphacelia, Gliocladium-like, or Phialophora-like anamorphic states were originally confined to Fescue and Lolium, species (Latch et. al., 1984; Morgan-Jones and Gams, 1982), however, recently the host range has been extended to Bromus and Poa (White and Cole, 1986).

The incidence of endophytes in tall fescue, perennial ryegrass and Italian ryegrass have been studied because these grasses are of prime importance in many countries. Tall fescue is extensively grown on 12-14 million hectares in the United States as a forage, turf and conservation grass (Siegel et. al., 1985), and the two ryegrasses are extensively grown in New Zealand and Europe.

It has been estimated that more than 90% of tall fescue pastures in the United States are infected with A coenophialum although new cultivars with a low incidence of endophyte have been developed (Siegel et. al., 1985).

2.4.2 ENDOPHYTIC FUNGI INFESTING GRASSES IN NEW ZEALAND

In New Zealand the common endophyte of perennial ryegrass (Lolium perenne) has been named Acremonium lolii (Latch, 1984). This species is morphologically similar to Acremonium coenophialum, a common endophyte of tall fescue and to the Acremonium-like anamorph of Epichloe typhina, a common endophyte of chewings fescue.

Both A. lolii and A. coenphialium are true endophytes, living vegetatively within the plant, being transferred in seed to the next host generation (Siegel et. al., 1987).

A lolii hyphae grow intracellularly during active growth periods of host plant being mainly confined to apical meristem tissue and aleurone layer of seeds during periods of dormancy (Neill, 1940; Lloyd, 1959).

2.4.3 LEVEL OF ACREMONIUM LOLII IN NEW ZEALAND GRASSES

Commercial seedlines of L. perenne, cultivars have variable levels of A. lolii, being dependent on parental source and storage process (Latch and Christensen, 1982; Scott, 1983).

In New Zealand Ellet, Yatsyn, Droughtmaster and 'Grasslands Marsden' perennial ryegrass cultivars all have high endophyte incidence in fresh seed lines while 'Grasslands Ruanui', 'Grasslands Ariki' and 'Grasslands Greenstone' have low incidence (Latch and Christenson, 1982; Thom et. al., 1987). 'Grasslands Nui' can have a high level of endophyte infection, although they are often lower and more variable (Pottinger et. al., 1985).

A.lolii has never been reported in Italian ryegrasses (L. Multiflorum) or short rotation ryegrasses (L. hybridum) such as 'Grasslands Manawa', although endophyte will infect these ryegrasses (Latch et. al., 1988). Latch et. al. (1988) recently detected Acremonium-like sp of fungus in Italian ryegrass, six annual ryegrass species and 'Grasslands Manawa'.

2.5 DISTRIBUTION OF ENDOPHYTE WITHIN PLANTS

The percentage of plants or seeds infected is termed the infection frequency and this is often used to rate pastures or seedlines as high or low endophyte. However, the distribution or concentration of mycelia varies throughout the plant.

Higher concentrations of hyphae occur in the leaf sheath than in leaf blades of vegetative tillers (Musgrave and Fletcher, 1984). It is found throughout the reproductive tiller, albeit in concentrations lower than in the live sheath component of vegetative tillers (Musgrave and Fletcher, 1984). The endophyte has not been isolated in roots (Latch et. al., 1984) although ELISA measurements gave slight positive results (Musgrave, 1984).

The distribution of A coenophialum in tall fescue appears to be similar to that of A lolii but the highest concentration has been found in the rachis (Burham and Bush, 1985).

2.6 ENDOPHYTE VIABILITY

2.6.1 SEED STORAGE EFFECTS ON VIABILITY

During the storage process endophyte mycelium in infected seed of perennial ryegrass and tall fescue die before the seed loses its capacity to germinate. Neill (1940) found endophyte mycelium within the ryegrass seed died if the seed was stored at room temperature for 18-24 months. Sowing this older seed will result in the establishment of endophyte free plants (Neill, 1940). This loss of viability during seed storage between harvest and sowing could explain why a survey by Latch (1985b) found that L. perenne seedlines with the highest incidence of endophytic fungi came from old permanent pastures when compared to pedigree lines, developed in plant breeding programmes.

However, seed which has been stored commercially for two and a half years has also been found with viable endophyte and therefore storage conditions may be important. Endophytes in infected seed have been found to remain viable for at least 15 years if the seed is stored at a temperature of 0-5°C and at low humidity (Latch, 1983), while seed in commercial stores frequently lose viability after two years (Latch and Christensen, 1982).

2.6.2 HOT WATER AND FUNGICIDIE EFFECTS ON VIABILITY

Endophyte free seed can be obtained by both fungicide treatment and hot water treatment. Soaking seeds of perennial ryegrass in water at 57°C for 15 minutes killed endophytes within them, but seed germination was reduced (Latch and Christensen, 1982).

Treating seed with fungicide will kill mycelium within the seed. The systematic fungicides procloraz and propiconazole, applied at rates of 1 kg/kg seed resulted in endophyte free seedlings (Latch and Christensen, 1982). However, these fungicides can be slightly phytotoxic. Endophyte mycellium can be killed in ryegrass by drenching benomyl into the root zone (Latch and Christensen, 1982).

2.7 GRASS - FUNGUS INTERACTIONS

2.7.1 THE CASE FOR MUTUALISM

Endophytic fungi infecting grasses are biotrophic; that is, they obtain all their basic nutritional needs from living tissue (Siegel et. al., 1987). Mutualism has been defined as a close association between members of different species that benefits both. The case for mutualism is clear but whether total separation of fungus from host grasses will affect survival of grasses in different environments is not known.

2.7.2 BENEFITS TO THE FUNGUS

Intercellular growth of Acremonium lolii is distributed unequally in the plant, with highest concentrations found in leaf sheaths, seeds and crown (Keogh, 1986). Although these regions can act as 'sinks' for the accumulation of soluble nitrogenous and carbohydrate substrate (Bacon et. al., 1986) they would have to leak out of the plant cells into the intercellular spaces and fluids for uptake by the endophytes. It is unknown whether endophytes can induce leakage or have translocation mechanisms in unknown for plant product movement.

Data on in vitro rates of growth, pH, temperature optimum, utilisation of carbon and nitrogen sources is available for some endophytes (Latch et. al., 1984; Davis et. al., 1986) but it is difficult to extrapolate this to in vivo situations. Endophytes clearly benefit from association with hosts by nutrition, long term protection, improved dissemination (via seed), and survival.

2.7.3 BENEFITS TO THE HOST

The plant benefits are complex and include increased tolerance to stress in endophyte infected grasses (Siegel et. al. 1987). Stress tolerance is characterised as resistance by plants to insect attack, overgrazing by herbivores and by enhanced plant growth. All these tolerances are found in Acremonium infested plants but are more pronounced in A. lolii infested ryegrass than A. coenphialium infested tall fescue (Siegel, et. al., 1987).

A. lolii infected 'Grasslands Nui' perennial ryegrass plants grown under controlled conditions in New Zealand showed significant increases in drymatter yield (38%) total leaf area, tiller numbers, and growth of leaves, pseudostems and roots when compared to non infected plants (Latch et. al., 1985b). The shoot:root ratio was unaffected. Infection of 'Grasslands Ruanui' with Gliocladium-like sp had no significant effect on yield even though total leaf area was reduced by 19%

The mode of action of Acremonium infection on physiology and growth patterns is unknown. Siegel et. al. (1987) suggested in their endophyte review that members of the Clavipiticeae produce, or induce the host plant to produce, auxin-like plant growth regulators and/or plant growth inhibitors, or alter hormone metabolism.

There are conflicting reports in the literature on the effect of endophyte infection on tall fescue growth and physiology. Siegel et. al. (1985) reported no difference in drymatter, seed yield or stand persistence in plots of tall fescue that ranged from 7-75% of plants infected with A. coenophialum, while Read et. al. (1986) found that infected tall fescue produced more and had greater persistence than non-infected fescue under grazing management.

2.8 INTERACTIONS BETWEEN GRAZING ANIMALS AND ENDOPHYTE INFECTED GRASSES

2.8.1 ANIMAL HEALTH

Neill (1940) suggested that endophytic fungi cause grass toxicosis in grazing animals but it was only recently that A. coenophialum and A. lolii infections of tall fescue Bacon et. al. (1977) and perennial ryegrass (Fletcher and Harvey, 1981) have been implicated in the cause of fescue toxicosis and ryegrass staggers respectively.

Ryegrass staggers is a temporary nervous disorder caused by animals ingesting A. lolii infected perennial ryegrass pastures (Mortimer, 1983). It generally occurs in the later summer/autumn period, 7-14 days after animals have been on toxic pastures (Mortimer et. al. (1982). The symptoms of mild attacks are slight to marked trembling of the neck, shoulder and flank muscles after exercise while severe attacks are characterised by marked uncoordination, a staggering gait and tetanic spasms (Mortimer, 1983). When disturbed, animals run with a stiff gait, stagger, fall and suffer muscular spasms (Mortimer, 1983).

Animals affected with ryegrass staggers have lower daily weight gains and produce less milk, perhaps attributable to reduced herbage intake (Fletcher and Barrel, 1984).

The prime suspect causative agents of ryegrass staggers are the neurotoxins lolitrem A and lolitrem B (Gallagher et. al., 1982, 1984, 1985). The major lolitrem neurotoxin, lolitrem B, is a lipophilic complex substituted - indole compound with a molecular weight of 685 and formula $C_{42}H_{55}No_7$ (Prestige and Gallagher, 1985). The lolitrems have not yet been reported to be produced by A. lolii in culture, but related tremorgenic toxins, penitrems, janithetrems and alfatrem are of fungal origin (Gallagher et. al., 1985).

Ryegrass staggers is most pronounced in summer-early autumn coinciding with peak endophyte growth within hosts and summer drought. Reduced pasture growth causes animals to ingest leaf sheaths and inflorescences where most endophyte is found (Keogh, 1983). Animals recover when transferred to endophyte free pasture (Mortimer, 1983).

2.8.2 OTHER EFFECTS ON ANIMALS

In addition to causing ryegrass staggers, infected grass can have subclinical effects depressing sheep liveweight gains during spring and reducing serum prolactin levels in sheep (Fletcher, 1986; Fletcher and Barrell, 1984). Similarly cattle feeding on forage from endophyte infested tall fescue had lower daily weight gains and lower milk production than cattle feeding on endophyte free tall fescue or tall fescue infested with low levels of endophyte (Siegel et. al., 1985). Infected ryegrass is also less palatable to stock (Hunt and Easton, 1989).

2.9 INSECT RESISTANCE DUE TO ENDOPHYTE

A. lolii confers resistance to Argentine stem weevil (Listronotus bonariensis) (ASW) in infected plants (Prestidge et. al., 1982) and seedlings (Stewart, 1985). Even non-viable endophyte may confer temporary seedling protection (Stewart, 1985). Oviposition is reduced (Gaynor and Hunt, 1983), larval survival is poorer and feeding is deterred (Barker et. al., 1984). The compounds responsible for ASW resistance in A. lolii infected perennial ryegrass have been identified as the peramine alkaloid (Rowan and Gaynor, 1986) and the lolitrem neurotoxin (Prestidge et. al., 1985).

2.10 EFFECT OF RYEGRASS CONTAINING ENDOPHYTE, ON THE PERFORMANCE OF ASSOCIATED WHITE CLOVER

Sutherland and Hoglund (1989) examined the effect of ryegrass containing the endophyte A. lolii on white clover performance. Irrespective of defoliation methods more clover seedlings survived under -E ryegrass than +E ryegrass. The dominant contrast was an inverse competitive relationship between gross yield and clover seedling survival. However, a further component of reduced survival with +E ryegrass was unexplained and was attributed to an allelopathic effect. This allelopathic effect on white clover germination was supported by Pederson (1986) (in Sutherland and Hoglund, 1989) working with tall fescue infected with endophyte. The suppression of clover growth and density may occur to the extent that soil nitrogen levels are lowered sufficiently to reduce subsequent crop yields (Sutherland and Hoglund, 1989).

2.11 METHODS OF ENDOPHYTE DETECTION

Endophytes can be detected in plants by staining the mycelium in leaf sheaths, stems and seeds (Bacon et. al., 1986), by enzyme-linked immunosorbent assay (ELISA) (Johnson et. al., 1982), by culturing on artificial media or by insect bioassay (Johnson et. al., 1985).

2.11.1 STAINING

Inspection of stained sheath tissue by microscope is frequently used to detect infection, often with difficult stains (Latch and Christenson, 1982; Saha et. al., 1988). The staining method clearly shows the coarse, mostly unbranched, convoluted intercellular nature of the mycelium. In seed, dead mycelium can not be distinguished from living mycelium, and in order to demonstrate endophyte viability, seeds must be germinated and three to four week old seedlings examined for mycelium (Latch et. al., 1984). A staining method to differentiate between dead and live mycelium would be of great benefit. A staining method to differentiate between dead and live mycelium would be beneficial. A quantitative assessment can be made by counting hyphae or scoring mycelium density (Harvey et. al., 1982).

2.11.2 ENZYME LINKED IMMUNOSORBANT ASSAY

Antisera have been prepared to cultures of A. coenophialum (Johnson et. al., 1982) and A. lolii (Musgrave, 1984), and are used in an enzyme linked immunosorbent assay (ELISA) to detect antigens of the fungi in tall fescue and perennial ryegrass. ELISA based on antibodies produced against mycelia does not differentiate between A. coenphialum, A. lolii or Acremonium-like species in Italian ryegrass, (Johnson et. al., 1985; Musgrave, 1984). In addition it does not differentiate between viable and non viable mycelium (Siegel et. al., 1987). While the ELISA technique is useful for determining the location and quantity of mycelium in infected plants and for detecting endophyte mycelium in seeds (Musgrave et. al., 1986) difficulties have been experienced in using the technique and this has led to an increased use of staining.

2.11.3 CULTURE

When it is difficult to identify endophyte in situ fungal endophytes can be cultured on artificial media, (Bacon et. al., 1977). However, this is not used as a routine identification method as endophytes grow slowly and are easily overrun by contaminating fungi and bacteria. It is relatively easy to culture and isolate endophytes from leaf sheaths, nodes, and stem pith (Bacon et. al., 1977; Latch and Christensen, 1982) but not seeds where heavy contamination of saprophytic fungi occurs.

2.11.4 INSECTS

Preference for endophyte free tall fescue and perennial ryegrass was shown by several species of aphids (Johnson et. al., 1985; Latch et. al., 1985a). This finding led Latch et. al. (1985a), to advocate the use of Rhopalosiphum padi as a bioassay to detect tall fescue plants infected with A. coenophialum and for large scale, rapid screening of endophyte in seedlings and mature plants.

CHAPTER 3
REVIEW OF LITERATURE

PART II : DIET SELECTION BY GRAZING ANIMALS

3.0 INTRODUCTION

Hodgson (1979) defined diet selection by grazing animals as the removal of some sward component(s) - plants or plant parts - rather than others. It is a function of preference, modified by the opportunity for selection, which is determined by the relative proportions of the preferred components in the sward, and their distribution within the canopy. Preference describes the discrimination exerted by animals between areas of sward or the components of a sward canopy, and between or within samples of cut herbage (Hodgson, 1979). The separation of preferences and opportunity is difficult as freedom of choice seldom exists. However, the effects of sward composition and structure on diet selection and differences in diet selection between ruminant species can be considered using the concept of preference and opportunity (Hodgson, 1982).

The selection of dietry components or euphagic behaviour is the dominant response when herbivores are presented a choice of potential forage. The act of selection is indeed one of the most important interfaces between pastoral producers and consumers.

The complex interactions between the animals and their environment, which results in the selection of a particular diet, has previously been extensively reviewed by Arnold (1964a, 1964b, 1981) and Hughes (1975). This chapter will review diet selection and grazing preference by animals.

3.1 PHYSIOLOGICAL FACTORS IN SELECTION

3.1.1 THE ROLE OF THE SENSES

The modalities of sensation observed in the nervous system of animals have been classified as tactile, temperature, kinesthetic, pain, auditory, visual, vestibular, gustory and olfactory (Breazile, 1971).

Of these Brobeck (1957) has suggested that intake facilitation or inhibitor responses to food on offer are associated with neutral pathways responsible to visual, olfactory, auditory, tactile gustatory and in addition, enteroreceptive stimulation.

Once the threshold of individual peripheral receptors to particular sensory modalities are reached, action potentials are generated in proportion to the intensity of the stimulus.

Interaction between and within modalities may exist, with the possibility that an unfavourable stimulus from one set of receptors overides a favourable stimulus from another set or intensifies the response to an existing unfavourable stimulus (Kennedy and Holgate, 1971). Hormonal concentrations related to physiologic conditions may also alter the intensity of response to neural stimuli (McManus et. al., 1968). The nervous system monitors, summarises, evaluates and quantifies the physiologic and environmental factors inherent in the animal thus initiating the logical reflexes of attention, approach, examination and ultimate selection or rejection of herbage (Brobeck, 1957).

3.1.2 SELECTION RESPONSES TO VISUAL STIMULI

Primarily, sight allows sheep to orientate themselves within their environment (Crofton, 1958). Although the visual sense has been observed to help sheep recognise plants it has previously experienced it appears that it is of little use for differentiating between plants with similar morphological characteristics, or between different plant parts (Arnold, 1981).

3.1.3 SELECTION RESPONSES TO TACTILE STIMULI

According to Davis (1925) harshness and hairiness in plants encourages rejection while succulance encourages acceptance. Recognition of this variability by neutral pressure receptors leads to selection of plants that give favourable stimuli (Arnold, 1966a). Consequently sheep retaining a tactile sense ate a less-steamy diet higher in carbohydrates than surgically non-tactile sheep (Krueger and Laycock, 1971). Arnold (1966) also hypothesized that this selection for low density green plant material, caused sheep to unconsciously select herbage of high nutritional quality and digestibility.

3.1.4 GUSTATORY AND OLFACTORY RESPONSES

Krueger and Laycock (1971) viewed taste as the most important sense in diet selection. Bell (1959) considered that it had protective as well as nutritive significance. Taste response is associated with the reaction of chemoreceptors with the papillae of the tongue to the threshold concentrations of the four primary taste modalities: sweet, sour, salty, and bitter (Bell and Kitchell, 1966). Goatcher and Church (1970a) showed the degree of taste response may be affected by the nature and temperature of the taste medium, visual and positional cues, age, disease, nutritional deficits, genetic constitution, and perhaps sex and psychological factors. The same authors (1970b) also found that cattle and goats compared to sheep required relatively high levels of taste stimulation before responding. The bitter taste caused the most adverse response. Plice (1951) demonstrated that the sweet taste was the dominant preferred taste. However, preference for a particular plant may not be due to animals selecting some attractive characteristic (taste) but rather to their selecting against the obnoxious characteristics of other plants (Arnold, 1981).

There are few reported studies relating the chemical composition of plants to their olfactory responses by, or palatability to animals (using the term palatability in the strict sense of relating to pleasant taste).

3.1.5 THE INFLUENCE OF AGE, BREED AND PHYSIOLOGICAL STATUS ON DIET SELECTION

Generally only small unimportant differences were found in the diet selected by sheep and goats of the same breed but different age (Hughes et. al., 1984). However, Arnold (1981) reported that at five months of age sheep had a diet that was higher in digestibility and in nitrogen content and lower in fibre content than that of older sheep. This may have been the consequence of the lambs small jaws which sllowed it to choose more precisely than the older sheep. Arnold and Hill (1972) noted that differences in preference existed amongst four breeds of sheep, and this was related to taste preferences.

Changes in physiological condition such as pregnancy and lactation do not themselves appear to affect the diet preferences of sheep. For example, Cook et. al. (1961) found no significant difference between digestibility of the diets of lactating and non-lactating ewes. Similarly, McManus, Arnold and Ball (1968) noted only small increases in the intake of soluble carbohydrate by ewes liberally fed during lactation.

3.2 ANIMAL FACTORS IN SELECTION

3.2.1 DIFFERENCES BETWEEN ANIMAL SPECIES IN DIET SELECTION

Genera of livestock differ in their preference for certain types of herbage. Linnaeus (quoted in Tribe and Gorden, 1950) offered 618 different plants both singly and in mixtures to sheep, cattle, goats, horses and pigs, discovering that in many cases, while animals of one or two different genera would accept a particular plant, those of another genus would reject it.

Hunt and Hay (1990) reported that deer show preference for low oestrogen red clover, calves prefer cocksfoot and horses prefer prairie grass. Sheep graze white clover in proportion to that on offer while goats reject white clover (Clark et. al., 1982).

Sheep graze deeper into sward canopy than cattle and select a diet containing higher proportions of live leaf and broad - leafed plants (Hodgson and Grant, 1980).

Selection differences may reflect differences in anatomy of mouthpaths (Van Dyne et. al., 1981). Cattle have no upper incisors and use the tongue to aid prehension. Because of the structure of the jaw they can seldom graze closer than 12mm from the soil (Van Dyne et. al., 1980). Sheep have a cleft upper lip which permits them to graze closer to the soil surface than cattle. The lips, the lower incisor teeth and dental pad are used in grazing (Van Dyne et. al., 1980). Goats have a mobile upper lip and prehensile tongue and thus can graze herbage as short as sheep. The goats ability to stand or climb on hind legs will also aid in diet selection. Diet differences between species may thus be taken to represent selection capabilities rather than selection drive. In some cases different diet selection may reflect species or size differences in the physical ability of animals to reach some components of particularly impenetrable swards (Hodgson, 1982).

3.2.2 DIFFERENCES IN DIET SELECTION BETWEEN INDIVIDUAL ANIMALS

Substantial differences exist between individuals of one genus. Arnold (1964) reported that the grass proportion of total intake for individual sheep could vary between 10 and 80%. Further, Van Dyne and Heady (1965) found between-sheep variation in the botanical composition of diet to be greater than within-day or between-day variation. Thus, although a population of sheep may indicate an average rating of preference among plants on offer, individual choice may vary widely either side of the mode. Therefore, there is the need for a reasonable number of animals in flocks or herds used in preference studies.

3.2.3 THE INFLUENCE OF PREVIOUS EXPERIENCE ON SELECTION

Robards (1965) examined the acceptability to sheep of cultivars of Phalaris finding that one cultivar became more acceptable as the grazing period increased, regardless of grazing pressure. The initial rejection was interpreted as an unfamiliarity with the plant as forage. Similar reactions by sheep to sweet vernal, and Yorkshire fog are reported (Jones, 1952). Arnold (1964a) stated that differences between sheep in how they accepted unpopular species slowly disappeared with at least one month elapsing before all his sheep would eat such generally disliked species as Stipa hyalina and Eragrostis curvala. He concluded that previous grazing experience had a strong influence on the preference ranking of species of plants.

3.2.4 NUTRITIONAL WISDOM

The survival of an animal species must indicate that sensory responses have been developed to give adequate nutrition. Domestic species are undoubtedly subject by man to sensory stimuli outside their inherent sensitivity range, which may explain why sheep, but not kangaroos in Western Australia eat the lethal plant Gastrolobium whenever they get the chance (Arnold, 1981).

Sheep may select areas of pasture high in phosphorous but will not correct a phosphate deficiency when given the chance (Arnold, 1981). Ozanne and Howes (1971) observed the selection by sheep of areas of high phosphate pasture, but these areas were associated with low phenol content. Whether the response was to phenol or phosphate was unknown. Sodium (Na) deficiency is rectified when Na is made available to sheep and preferential selection of herbage species high in Na has been observed although it appears they cannot detect levels less than 0.1% in herbage and will not select for Na below this level (Arnold, 1964b). Thus care must be taken when judging if there is motivation for selective grazing based on nutritional wisdom. Another example of this is the selection of green leaf in preference to dead (Geenty, 1983). Although this material has higher nutrient content and digestibility the selection may be based on ease of harvest and accessibility rather than nutritional wisdom.

It would seem that innate reactions determine selection by grazing animals. Thus innate food selection directed toward optimal nutrition and avoiding intoxication may occur in conjunction with minimising unpleasantness or maximising pleasantness of olfactory and other sensations. This however, does not manifest when the mechanics of grazing result in the removal of the top canopy of feed first, even when the highest quality feed may be in the base of pasture.

3.3 EXTERNAL FACTORS IN SELECTION

3.3.1 DIURNAL VARIATION IN DIET SELECTION

The daily routine of an animal is determined by the diurnal pattern of grazing, with rumination, periods of rest and drinking being fitted into intervals when the animal is not grazing. Major grazing periods begin near dawn and again in late afternoon in both northern (Hughes and Reid, 1952) and southern (Arnold, 1962) hemispheres. As days shorten, the breaks between grazing also descrease until in mid-winter, in latitudes of 20° or more, grazing is almost continuous during daylight (Arnold, 1981).

Temperature can also influence grazing behaviour. Indications are that when daily maximum temperatures are <15°C little night grazing is done, but when they are high, >25°C, night grazing varies from 0 to 70% of total grazing time (Arnold, 1981). Arnold (1964b) reported that as daily grazing time increased selectivity also increased.

Diet selection by sheep and cattle alters during the day (Arnold, 1981). Variation is most pronounced on rangelands or high country where diets alter with location change. Conflicting evidence exists as to whether the diurnal variations are consistently reflecting feedback mechanisms or inconsistent reflection of satiation. Arnold (1964b) found inconsistent within day variation in diet, while Langlands (1967) found N content of diet increased as the day progressed. Ohioha et. al. (1970) (quoted in Arnold, 1981) found that the diet of cattle contained more grass than forbs in the morning and the converse in the evening. This suggests that because grass was more accessible, selection was influenced by the animals desire to eat quickly during the morning grazing period.

3.3.2 INFLUENCE OF SOIL PHYSICAL AND CHEMICAL STATUS ON SELECTION

Harlan (1956) noted that the acceptability level of plants varied with soil fertility. This occurred to the extent that Bermuda grass (Cynodon dactylon) was eaten readily on fertile soils but rejected on infertile soils. Soil moisture status is also of importance. Plants growing at field capacity are likely to be more succulent, and accessible due to erect, upright growth habit than those suffering soil moisture deficits (Hughes, 1975).

Research suggests animals prefer plants fertilised with phosphorus (Plice, 1951; Ozanne and Howes, 1971). The mechanism of preference is considered to be influenced by the higher soluble carbohydrates (Plice, 1951) or low free phenol content (Ozanne and Howes, 1971) in phosphorus fertilised plants.

Livestock select urine-patch in preference to inter-urine patch and dung patch herbage (Keogh, 1986). Under continuous stocking this was expressed as a higher frequency and greater intensity of defoliation of herbage at urine patch sites (Keogh, 1973). Under rotational grazing livestock remove a greater proportion of material present at urine patches than at inter-excreta sites (Keogh, 1973). White clover incidence also influences feeding behaviour. Inter-excreta areas containing white clover are grazed in preference to similar ryegrass dominant areas devoid of white clover (Keogh, 1973). Ryegrass present at sites containing white clover is also grazed much closer that ryegrass at either urine-patch or inter-excreta sites (Keogh, 1986).

3.3.3 THE INFLUENCE OF CLIMATE ON SELECTION

Climatic stress can affect the morphology of plants, their nutritive value, moisture content, and relative acceptability to grazing animals (Hughes, 1975). High summer temperatures accentuate the plant characteristics of cuticle and epidermal thickening, hairiness, and spine-form leaf adaptations associated with avoidance of transpiration stress. The presence of these features in plants tends to decrease their acceptability (Heady, 1964).

3.4 INFLUENCE OF GRAZING INTENSITY ON SELECTION

The grazing pressure on a paddock will increase if the stocking rate remains constant, but pasture consumption exceeds the rate of pasture production. The choice of plants or plant parts available to the animal decreases as grazing pressure increases. Previously unpreferred plants are more likely to be eaten as preferred plants become scarce. Consequently, there is a successional change in the diet selected (Hughes, 1975).

Cook et. al. (1953) reported that as the opportunity for selection between plants decreases there may be an increase in the proportion of fibre and lignin in the forage intake. Arnold et. al. (1966b) found that the proportion of protein in the diet fell initially with increased grazing pressure but later increased as pasture growth improved with more intensive grazing.

3.5 DIET SELECTION AND DIGESTIBILITY

Although numerous attempts have been made to predict the diet selected from the relative digestibility of the offered feed it seems unlikely that selection is based on digestibility. Digestibility is likely to be correlated with other factors that influence diet selection.

Plants of high digestibility are likely to have characteristics such as a high proportion of non-structural carbohydrates, proteins, and organic acids (Van Soest, 1968). Such characteristics make them more acceptable to sheep than plants of low digestibility. Alternatively, high lignin content (Corbett, 1969) in low digestibility plants could also influence acceptability.

The proportion of lignin and structural polysaccharides increases with age, and is more pronounced in later formed tissues such as culms and stems (Hughes, 1975). According to Heady (1964a) this increase parallels decreasing acceptability to the grazing animal. However, since the rate of increase of structural polysaccharides and lignin varies between plant parts, the increase in the maturing plant as a whole will depend not only on changes in the level in the leaf, but also on the relatively greater changes and higher level in the developing stem. Consequently, lignin level, cellulose level, digestibility and acceptability change less in the plants kept by periodic defoliation in vegetative stage, than those allowed to mature.

3.5.1 DIET SELECTION AND CHEMICAL COMPONENTS

Numerous attempts have been made to relate diet selection to the approximate composition of plants. But it is not possible for the animals to recognise such things as 'soluble carbohydrates', 'nitrogen', 'crude fibre', 'energy' or 'ash' as these fractions do not exist in this form at molecular level in plant (Arnold, 1981). Where correlations are found, they must relate to specific components or some physical properties of the plant. For example, ash with Na and K. Crude fibre levels in diet could be related to ease of harvesting.

Research work that aims to find correlations between a single compound and preference is often misinterpreted. The lack of a correlation does not mean that the factor is not involved in diet selection because selection is a multi-dimensional system. Instead it indicates that the compound is not of great importance. Conversely correlations between a single compound and preference is not proof that it is the main component influencing selection. Animals cannot select a food objectively because it is high in 'energy' or produces the best weight gain (Arnold, 1981).

However, some broad relationships have been shown to exist. Preference ratings amongst strains of Serica lespedeza have been shown to be related to tannin content (Arnold, 1981). Quinolizidine alkaloids present in the genus Lupins influence the consumption of green lupins by sheep and cattle (Bush et. al., 1972, quoted in Arnold, 1981). Other reports are of preference being correlated with fat content or either extracts (Arnold, 1981).

These are all single classes of compounds, but as stated animals respond to integrated effects of the stimuli from various classes of compounds. Jones and Barnes (1967) (quoted in Arnold, 1981) studied the integrated responses to a number of organic acids. Significant correlations occurred between preference and certain compounds, but no single variable accounted for more than 64% of variation in preferences. This illustrates an integrated response by grazing animals to plant characteristics.

3.6 THE INFLUENCE OF SWARD CHARACTERISTICS AND PLANT SPECIES ON DIET SELECTION

A typical mixed sward canopy in a vegetative stage of growth consists of an upper horizon consisting primarily of grass leaf lamina and a lower horizon consisting of leaf sheaths (aggregated into pseudostem) and dead tissue. Legumes, herbs and in mature swards true stems, are distributed throughout the canopy (Hodgson, 1979).

Separating independent effects of variations in gross sward characteristics such as herbage mass, sward height, pseudostem height, density and green leaf distribution on diet selection is difficult. Observations of gross sward characteristics probably do not provide an adequate description of vegetation as perceived by the grazing animal.

The relative proportions of the different plant species or components and their distribution in space will modify selection. The grazing process has been described as "movement in a horizontal plane and selection in a vertical plane" (Arnold, 1964b). This restrictive definition of the grazing process has since been modified into a two phase concept involving "site selection' and "bite selection" (Milne et. al., 1979).

3.6.1 SITE SELECTION

Site selection has been reported on a scale varying from several metres in set-stocked sheep on ryegrass dominant pasture in New Zealand (Keogh, 1973) to several kilometres for shorthorn cattle grazing rangeland in central Australia (Low et. al., 1981). Initially grazing site will be determined by the physical environment - slope, aspect, topography, distance to water, climate, animal tracks, shade availability, and subdivision (Arnold, 1981).

In Australia it is recognised that sheep paddocks on rangelend are most heavily utilised in the areas from which the prevailing winds come (Arnold, 1981). Arnold (1981) also reports variations in intensity of sheep grazing with elevation and rainfall in the mountains of North Wales.

Site selection is also influenced by more local factors. These include the distribution of green areas (Low et. al., 1981), differences in stage of growth of different plant communities (Arnold, 1981) and the patchy distribution of dung and urine. As yet the way these factors interact has not been fully understood (Hodgson, 1982).

3.6.2 BITE SELECTION IN RELATION TO SWARD STRUCTURE AND PLANT SPECIES

Bite selection is the choice of individual bites of herbage from the vegetation at a chosen site. The opportunity for bite selection reflects the heterogenous nature of natural pastures and the distribution of plant components in vertical and horizontal planes. Canopy structure may also be important. The open structure of annual tussock grasslands allows easy access to all levels of sward (Hodgson and Grant, 1980), whereas many temperate grasslands have short closed canopies that do not allow easy access (Hodgson, 1982).

Selection will depend on both preference contrasts between alternative components of swards and their distribution within the canopy (Hodgson, 1982). Sheep may graze relatively indiscriminantly at the surface of intensively managed Lolium perenne/Trifolium repens swards (Milne et. al., 1982), but selection for T repens growing close to base of indigenous Agrostis/Festuca swards can be more extreme (Hodgson and Grant, 1980).

Barthram and Grant (1984) have shown that sheep readily graze within the leafy horizon of a vegetative sward, but are reluctant to penetrate into lower horizons containing pseudostems and dead material. The depth of the leaf horizon appears to set an effective limit to the depth of bite. The depth appears to be reached for sheep in swards with leaf horizon, less than 20-30mm deep (Barthram and Grant, 1984), but in swards with a deeper leaf horizon there is a roughly proportional relationship between sward surface height and bite depth (Milne et. al., 1982).

L'Huillier et. al. (1984) stratified the sward into horizons and found green grass leaf was preferred and was a major determinant of the horizon grazed. Pseudostem content of diet was low and rejected by the grazing animal. Animals grazing leaves before stems is in part related to plant anatomy. It would be difficult to graze the stem without grazing the leaf, but it is possible to graze a leaf without grazing the stem. It is rare for animals to graze from the bottom of the pasture upwards in the sense of biting whole plants off at their bases.

L'Huillier et. al (1984) found that white clover was harvested by sheep in proportion to its presence in all horizons of sward indicating no diet selection, while Clark et. al. (1982), and Bootsma et. al. (1990) observed selection for white clover by sheep. The fact that L'Huillier et. al. (1984) reported no diet selection occurring implies that the vertical distribution of major dietary components influence white clover consumption rather than animal preference. To demonstrate diet selection unequivocally in mixed swards information is required on the degree of aggregation of grass and clover within the sward and the vertical distribution of grass leaf lamina relative to clover lamina and petiole.

Milne et. al. (1982) suggested that in intensively managed temperate swards the composition of the diet appeared to be the natural consequence of a largely unselective grazing habit superimposed on a stratified distribution of plant tissue. Milne et. al. (1982) predicted that animals would graze indiscriminantly at the surface with dietry components reflecting the proportion of component in that horizon. Variation in white clover proportion in diet was explained by variation in white clover proportion of grazed horizon. It should not be assumed that the existence of a difference between sward composition and composition of total herbage on offer is indicative of diet selection as diet selected may not differ from diet offered in each grazed horizon. However, the fact the the animal has chosen to graze at a particular site and particular horizon is a form of selection in itself.

Recently photographic techniques have been developed to assess pasture species preference of grazing animals (Hunt and Hay, 1989). These authors observed the preferences of horses, deer and calves for 16 grasses, herbs and legumes. 'Grasslands Matua' was the most prefered with horses spending twice as much time grazing Matua plots as most other grasses. Deer showed a clear preference for legumes and herbs, particularly low oestrogen red clover and calves showed a strong preference for grasses other than ryegrass. There is little literature availability on grazing preferences by sheep for different species.

3.6.3 THE INFLUENCE OF PSEUDOSTEM ON DIET SELECTION

The layers containing leaf sheath (pseudostem) inhibit grazing and limit the depth of the grazed layer, even when the consequence is a marked reduction in herbage intake. An impenetrable barrier was not present but inhibition was clearly effective (Barthram, 1980). This observation helps explain why herbage intake falls with a reduction in allowance or sward height, even in circumstances where substantial amounts of herbage remain ungrazed. In practical terms, this means that measurement of grazed height may not provide a good index of intake limitations unless they can be related to information about the distribution of pseudostem within the sward canopy.

Grazing inhibition due to pseudostem could be attributed to greater structural strength of stems when compared to leaves (Heady, 1975), proximity of soil surface (Arnold, 1960), higher microbial population in leaf sheaths e.g. Accemonium lolii (Keogh, 1986), and increased dead material (Barthram, 1980).

3.6.4 THE INFLUENCE OF HEIGHT AND DENSITY ON SELECTION

Generally on vegetative swards herbage intake increases with height although the relationship depends on whether height was measured on extended tiller (Allden and Whittaker, 1970) or as the height of the grazed surface (Hodgson, 1982). Higher intakes have been measured on spring swards compared to summer or autumn which may be due to the more erect spring swards encouraging greater bite depth.

Under field conditions it is difficult to separate the natural correlation that exists between height and density of pastures or to manipulate one factor independently. Therefore, confounding results may be obtained. Furthermore, plants of differing height often vary in stage of maturity and this may influence the rate of consumption (Black and Kennedy, 1984). Black and Kennedy (1984) overcame the problems by constructing artificial swards of differing height, density and spatial arrangement by placing tillers in holes drilled in wooden boards. The authors found that sheep generally preferred long swards to short swards and sparse swards to dense swards. When sheep were offered artificial swards for periods short enough not to induce satiation, the rate of intake was closely correlated to the intake per prehending bite which decreased with a reduction in sward height and tiller density. However, it was also affected by the spatial arrangement of tillers and thus more closely related to herbage mass per area effectively covered by one bite than herbage mass over the whole area.

Clark et. al. (unpublished) examined preference and intake components of cattle, sheep, and goats offered perennial ryegrass of differing height. All three species preferred grazing tall swards. Goats showed the most consistent preference for taller swards, followed by cattle, with sheep showing indifference to smaller height differences.

A common observation is the avoidance of tall patches in pasture of variable height. The findings of Clark et. al. (unpublished) and Black and Kenney (1984) would suggest that this is unlikely to be avoidance of tall pastures per se. Possible explanations for this are faecal contamination and maturity differences.

3.7 THE INFLUENCE OF POTENTIAL INTAKE RATE ON DIET SELECTION

Black and Kennedy (1984) suggested that potential intake rate (PIR) was a major determinant of the diet selected by sheep. The potential intake rate at which feed can be eaten is determined largely by the physical characteristics of the feed such as ease of fracture, particle size and water content, (Colebrook et. al., 1985); but the size of an animals mouth, its degree of satiation and physiological state may also be important (Hodgson, 1982).

The potential intake rate by sheep of feeds varies and for some feeds can be altered by changing physical characteristics such as particle length (Colebrook et. al., 1985; Black and Kennedy, 1984). There is a definite preference for feeds which can be eaten faster; the degree of discrimination between feeds being greater when the potential intake rate of feeds being compared is low (Black and Kenney, 1984). However, the same authors also found that the preference for some feeds; such as dried clover pasture, was less than would be expected from the potential intake rate at which the feeds are eaten and suggested acceptability factors such as taste, odour or feel are also important. Moseley and Antuna-Manendex (1989) developed a simple method to determine the rate of eating of a feed. This involved offering animals small consecutive meals following a fasting period and measuring the amount consumed in one minute intervals. The methodology provided a rapid method for the evaluation of intake characteristics of forages.

3.8 OPTIMAL FORAGING THEORY

If nutritive and sward characteristics are the main determinants of diet selection, then it follows that the relative rates of intake will strongly influence the animals foraging behaviour. This proposition is fundamental to the paradigm of optimal foraging, which has been the subject of much theoretical and empirical study. The optimal foraging theory predicts that animals will choose a diet to maximise the intake of some nutrient currency while minimising the cost of foraging and exposure to predators (Illius, 1986).

In the case of ruminants the most important nutrient currency is likely to be energy due to the correlation between the content of digestible organic matter in herbage tissues and contents of nitrogen and minerals. Selection of plant material with a low ? content of digestible organic matter in herbage tissues represents an avoidance of the less-digestible structural carbohydrates (Van Soest, 1968). Preference will be given for foods which give the highest intake per unit handling time (Illius, 1986).

If an animal is to make an informed choice, and select a preferred a diet, a knowledge of the offered diet is required. It cannot be assumed that animals have complete nutritional knowledge, to optimise foraging in a variable environment and hence must sample to ascertain the relative values of food sources. This would explain why diets selected are often sub-optimal in comparison with the linear programming solution to the problem of the best nutrient mix (Illius, 1986).

Clark et. al. (unpublished) noted sub-optimal grazing behaviour of sheep as they spent some time grazing shorter swards when higher intake could be gained on taller swards. The greatest proportion of intake and the highest proportion of time spent grazing tall swards in tall-short height comparisons occurred when the difference between sward height was greatest. This suggests that when there are large differences in the possible intake that can be achieved, selection based close to optimal foraging theory will occur.

3.9 GRAZING BEHAVIOUR

The grazing intake of an animal is the result of the size of bite, number of bites per unit time and the amount of time an animal spends grazing (Allden and Whittaker, 1970). It is often expressed by the following formula:

$$I = IB \times RB \times GT$$

where I = intake
IB = intake per bite
RB = rate of biting
GT = grazing time

Sward characteristics (e.g. green leaf distribution dead material content) would influence intake per bite and rate of biting with the animal modifying its response by altering grazing time and perhaps rate of biting. Of these behavioural parameters intake per bite appears must sensitive - varying up to ten-fold (Hodgson, 1981), while grazing time and rate of biting are limited to two-fold changes (Hodgson, 1982).

Plate 1 View of paddock 6 from raised tower during trial 2 (May 14-21) indicating trial layout and urine-patch response

CHAPTER 4
MATERIALS AND METHODS

4.1 GRAZING TRIAL

4.1.1 EXPERIMENTAL SITE AND DESIGN

Research plots were established at the Plant Science Department Iversen Field Research Area at Lincoln University, Canterbury. Two blocks (0.2 ha paddocks) each containing three replicates of three grass treatments (main plot treatments) were sown in October 1989. These plots were used for trial 1 (Section 4.2) and trial 2 (Section 4.3).

The main plot treatments were:

i. 'Grasslands Roa' tall fescue (Festuca arundinacea) plus 'Grasslands Pitau' white clover (Trifolium repens L.).

ii. Endophyte infected (+E) 'Grasslands Nui' perennial ryegrass (Lolium perenne L.) plus 'Grasslands Pitau' white clover.

iii. 'Grasslands Nui' perennial ryegrass without endophyte infection (-E) plus 'Grasslands Pitau' white clover.

Plots (7m x 35m) were arranged side by side across the block. Design was arranged so that the same main plot treatments were never next to each other. An aerial view of one block from an observation tower is shown in Plate 1.

The soil type was a Wakanui silt loam which had previously been under sheep grazing trials for several years.

4.1.2 PRE-TRIAL MANAGEMENT

The plots of +E and -E perennial ryegrass/white clover and tall fescue/white clover were sown in October 1989 and grazed rotationally from December to February at variable stocking rates with Coopworth ewe hoggets. The area was irrigated to maintain the potential soil water deficit at less than 25mm.

Plots were trimmed on 27 February 1990, using a flail type mower to a uniform height of 5cm. From 13 March to 18 March 1990, swards were grazed with 40 Romney ewe hoggets from an initial herbage mass of 2-3000 kg DM/ha to a residual of 4-800 kg DM/ha. Mowing and grazing were necessary because pasture mass, height and structure of swards were dissimilar.

The aim was to obtain a similar pasture mass, dead material content, and height for all three grass treatments at the commencement of the experimental grazing. However, the different intensities of grazing and differential rates of recovery created dissimilar pasture mass at the commencement of the experimental grazing. To allow visual discrimination between plots the perimeter drill row of each plot was removed by spraying with 'Buster' on 24 March 1990 (see Plate 1 and 3).

4.2 FIRST EXPERIMENTAL GRAZING (3-12 APRIL) - TRIAL 1

The sheep used for grazing were Coopworth ewe hoggets from the Lincoln University Research Farm. Fifteen sheep were introduced into each block on 3 April 1990 and allowed to graze till 12 April 1990. The number of sheep introduced was calculated from visual estimates of the feed available and feed requirements of sheep. The aim was to reduce the average pasture mass in each block by 1000 kg DM/ha in a ten day period of grazing.

4.2.1 PASTURE MASS

Pre-grazing (3 April 1990) and post-grazing (12 April 1990) pasture mass were measured for each grass treatment. Two randomly placed $0.1m^2$ quadrats in each plot were cut to ground level. All samples were dried at 80 °C for 24-48 hours and were then weighed.

4.2.2 GRASS AND CLOVER LEAF HEIGHT

Measurements of the height of grass leaves and white clover leaves were made on April 3, 5, 7, 9 and 12 1990. Height measurements were made using a sward height stick. As the perspex disk was lowered onto the sward the height of the third grass leaf touched and the first white clover leaf touched were measured. The heights of ten grass leaves and clover leaves were measured at random positions in each plot.

Leaf extension rate for each grass treatment was measured in protective cages. One cage (1·2x3m) was placed on one single plot of each grass treatment per block and the height of ten grass leaves and ten white clover leaves measured (using same technique as described above) prior to and after grazing. Average leaf extension rate per day was then calculated and grass leaf and white clover leaf height corrected for this growth.

4.2.3 PASTURE COVER

Pasture cover percentage was estimated on April 3, 5, 7, 9 and 12 1990. Four cover categories were recognised: green grass, clover, dead material and litter, and bare ground. A point analyser with 20 points was used to determine the cover percentage. The point analyser was placed randomly at right angles across the seed drill rows in four positions per plot. Twenty first hits were recorded at each position per plot (80 hits per plot). Percentage cover was then calculated.

4.2.4 PSEUDOSTEM HEIGHT

Pseudostem height was recorded before (3 April) and after (12 April) grazing. Thirty randomly selected tillers per grass treatment were cut to ground level with a knife and the height of pseudostem measured with a ruler. Pseudostem height was described as the height of the base of the first leaf of the tiller above ground level.

4.2.5 GRAZING BEHAVIOUR OBSERVATIONS

Following the introduction of sheep into the plots their preferences were recorded by visual observations. Visual observations were made from a raised tower (5m high), located mid-way between the two blocks (see Plate 1 for view of one plot from tower). The number of animals grazing each species was recorded at two minute intervals for a one hour period (30 recordings). Only sheep that could be seen actively grazing were recorded. Sheep that were sitting/lying down and those standing on plots but not grazing were not recorded as grazing animals. Observations were made on April 3(am), 4(am), 5(am), 6(am), 7(am), 9(pm) and 12(am) 1990. Morning (am) observations were made between 8.00 am and 10.00 am, while evening (pm) observations were made between 4.00 pm and 6.00 pm.

4.3 SECOND EXPERIMENTAL GRAZING (MAY 14-21) - TRIAL 2

4.3.1 EXPERIMENTAL DESIGN AND GRAZING ANIMALS

The two blocks (paddocks) described in Section 4.1.1 were used in a split plot design. The three replicates of main plot treatments in each block were either cut with mower at 2.5 cm or left unmown on 27 April 1990 (subplots). Cut areas were approximately one-third the area of main plots. The sub-plots were further stratified visually into urine and non-urine patch areas (sub-sub plots) (See Plate 1 for indication of urine patch response). This was done on the base of colour. Dark green areas were designated urine patch sites while light green areas were designated non-urine patch sites. Twenty sheep were introduced into each block on 14 May 1990 and allowed to graze till 12 April 1990. The aim was to reduce pasture mass by 1000 kg DM/ha in an eight day grazing period.

4.3.2 GRASS LEAF HEIGHT

Measurements of the heights of grass leaves were made on May 14, 16, 17, 19 and 21 1990, by the method described in Section 4.2.2. The heights of eight grass leaves were measured in urine patches and non-urine patches of each sub-plot.

4.3.3 PSEUDOSTEM HEIGHT IN TALL FESCUE/RYEGRASS ASSOCIATIONS

The tall fescue - white clover plots were contaminated with +E ryegrass (50% tillers of ryegrass infected with endophyte) plants. The plants were established in the seed drill rows with few plants intermediate between rows indicating seed contamination. In order to examine the intensity of grazing within these associations pseudostem height was measured on 14 May 1990.

Eight clumps of each of pure tall fescue, pure ryegrass and tall fescue-ryegrass associations were identified per plot. For the pure clumps the height of one pseudostem of a tiller in that clump was measured with a ruler. For the mixed clump the height of the pseudostem was recorded for both one ryegrass and one tall fescue tillers per clump.

4.3.4 TILLER NUMBERS

Tiller densities were measured in urine and non-urine patches prior to grazing (14 May). One 0.01 m^2 quadrat was placed on a urine and non-urine patch in each grass treatment and tiller number counted.

4.3.5 PASTURE MASS

Pre-grazing (May 14) and post-grazing (May 21) pasture mass were measured. Two 0.1 m^2 quadrats were placed on urine and non-urine patches in mown and unmown subplots of each grass treatment and pasture cut to ground level. All samples were dried at 80°C for 24-48 hours and then weighed.

4.4 EATING RATE EXPERIMENT

4.4.1 EXPERIMENT 1

4.4.1.1 Experimental Animals

Nine-month-old Romney ewe hoggets (approximate liveweight = 25kg) were used to examine the effect of contrasting feed types on rate of eating. Eight animals were individually housed in metabolism crates. Prior to the commencement of the eating rate trial the sheep were fed ad-libitun a mixed diet of tall fescue, +E ryegrass, -E ryegrass and white clover.

4.4.1.2 Feed Types

The food types used were tall fescue, +E 'Grasslands Nui' ryegrass (70% endophyte infection) -E 'Grasslands Nui' ryegrass (0% endophyte infection) 'Grasslands Roa' tall fescue and 'Grasslands Pitau' white clover. The grass feeds were obtained from pure swards cut to 1 cm above ground level using a sickle bar mower. Ryegrass material was approximately 120 mm long which included 35 mm of pseudostem. Tall fescue material was approximately 100 mm long which included 30 mm of pseudostem. The clover forage was obtained from pure swards cut to 1 cm above ground level using a mower which caused chopping of leaf and petiole. Individual fresh samples (100g) were weighed out using an electronic balance. These were either used within two hours or stored at -10°C for up to 9 hours.

4.4.1.3 Measurement of Eating Rate

Animals were denied access to feed for nine hours before measuring eating rates at 0730 hr. The measurement of eating rates was carried out by offering consecutive meals of 100g of test feed and recording the length of time taken by sheep to consume each meal. Four forages were tested per sheep. The different feeds were fed in a systematic design amongst eight consecutive meals. The experiment was replicated the following day.

4.4.2 EXPERIMENT 2

4.4.2.1 Experimental Animals

In a second experiment, six of the eight nine-month-old Romney ewe hoggets (Live weight = 25kg) used in experiment 1 were used to examine the effect of contrasting grass components on eating rate. Animals were maintained in the same metabolism crates as in experiment 1.

4.4.2.2 Feed Types

The forages used were 'Grasslands Roa' tall fescue (leaf and pseudostem), +E 'Grasslands Nui' ryegrass (70% endophyte infection) (leaf and pseudostem) and -E 'Grasslands Nui' ryegrass (0% infection) (leaf and pseudostem). Leaf material of the three grasses was obtained from pure swards by cutting with a sickle bar mower set to cut just above pseudostem height (35 mm). Once the leaf material was removed, pseudostem material was obtained by cutting the remaining material of the same sward to 1.5cm above ground level using a sickle bar mower. Fresh samples were weighed out using an electronic balance.

4.4.2.3 Measurement of Eating Rate

All animals were denied access to feed for nine hours before measuring eating rates at 0730 hr as described in Section

4.4.1.3. The six feed types were tested once on each sheep on two successive days. Sheep received a small meal following the first measurement of eating rate.

4.4.3 DRY MATTER % DETERMINATION

Fresh subsamples (200g) of all the feeds used in the measurement of eating rate were taken when samples were cut. Fresh and dry herbage was weighed on electronic balance in laboratory. Fresh herbage was dried in a force draught oven at 80°C for 24 hours. Dry matter percentage (DM%) was then determined.

4.5 ASHLEY DENE GRAZING TRIAL

4.5.1 EXPERIMENTAL DESIGN

Four replicates of each of eight grasses or herbs were sown at Ashley Dene in August 1989, in plots 30m x 9m. Plots were arranged side-by-side, each replicate block occupying one quarter of the grid.

4.5.2 ANIMALS AND GRAZING

On 25 September 1990, 170 ewes with lambs at foot were introduced into the plots and allowed to graze for ten days. Plots had previously been ungrazed since May 23, 1990.

4.5.3 GRASS HEIGHT MEASUREMENTS

Grass height was measured in only +E and -E ryegrass plots on September 25 and 29, and October 2 and 5 by the method described in Section 4.2.2. The heights of twenty grass leaves were measured at random positions in each plot.

4.6 ENDOPHYTE DETECTION

Endophyte fungi presence was detected by microscopic inspection of stained grass sheath tissue following the method described by Saha et. al. (1988). For grazing trials 1 and 2 twenty tillers collected at random from +E and -E ryegrass plots in each block were inspected, with presence or absence of hyphae being recorded. No quantitative assessment of mycelium density was made.

Endophyte detection in the eating rate experiment (Section 4.4) was done by collecting twenty tillers from pure swards of +E and -E ryegrass and noting whether hyphae were present or absent. At Ashley Dene endophyte incidence was recorded in twenty tillers collected at random from +E and -E ryegrass plots. In addition, the endophyte incidence was determined in twenty tillers collected from lax and hard grazed areas of a +E ryegrass plot.

4.7 STATISTICAL ANALYSIS

Results were analysed using the 'MINITAB' statistical package. Least significant differences (l.s.d. P=0.05) were applied where appropriate are included in Tables. Stand error of means are presented in graphs.

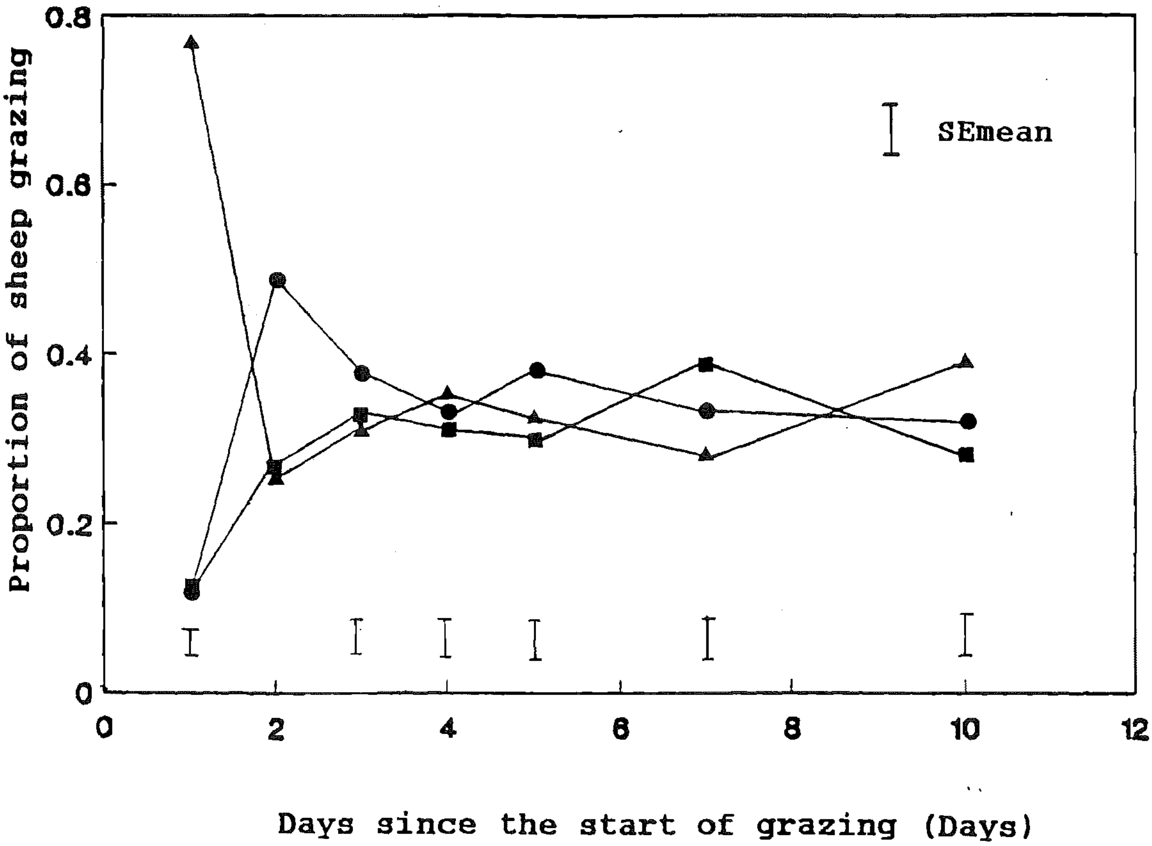


Figure 1 Proportion of sheep grazing each grass in one hour periods during trial 1 (April 3-12). (▲) tall fescue, (●) +E ryegrass, (■) -E ryegrass.

CHAPTER 5
RESULTS

5.1 TRIAL 1

5.1.1 PASTURE MASS

Pre-grazing pasture mass (kg DM/ha) of +E and -E ryegrass were similar. However, they were 40% greater than the pasture mass of tall fescue swards (Table 1). Post-grazing pasture mass (kg DM/ha) of +E and -E ryegrass were nearly twice those of tall fescue (Table 1) (Plate 2 a,b,c). More pasture (kg DM/ha) was removed from the -E ryegrass than the +E ryegrass or tall fescue.

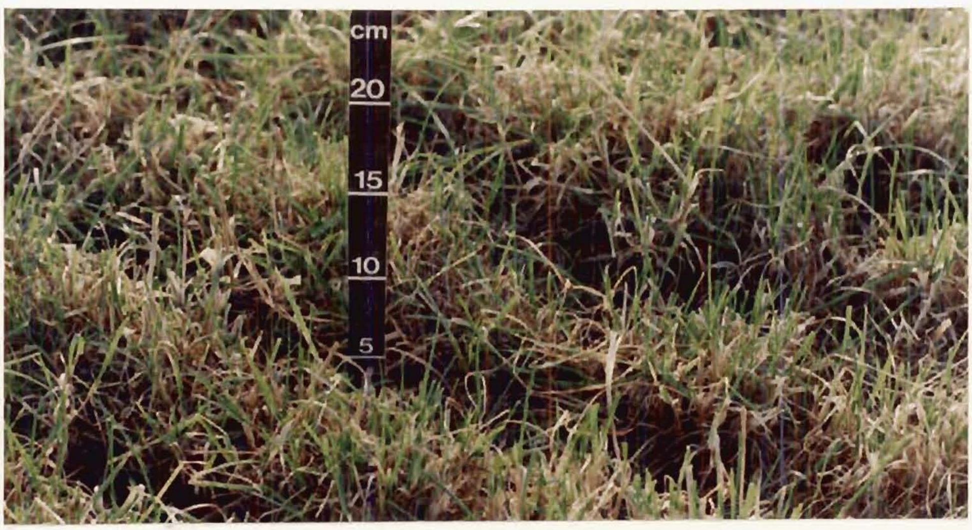
Table 1 Pre-grazing and post-grazing pasture mass of grasses for trial 1 (April 3-12)

	Pasture Mass (kg DM/ha)		
	Pre-graze	Post-graze	Change
tall fescue	1620	600	1020
+E ryegrass	2360	1260	1100
-E ryegrass	2240	1000	1240

5.1.2 OBSERVATIONS

The grazing preference of ewe hoggets over the nine days of grazing is shown in Figure 1. On day 1 of grazing (3 April) a very high proportion (76%) of ewe hoggets grazed tall fescue, while a low proportion (12%) grazed +E and -E ryegrass (Figure 1). The proportion of ewe hoggets grazing tall fescue declined rapidly on day 2 (4 April) and remained in the 30-40% range over the remainder of grazing. The proportion of ewe hoggets grazing +E ryegrass increased on day 2 (4 April) to a maximum of 49% but then declined gradually over the remainder of grazing. The proportion of ewe hoggets grazing -E ryegrass increased

a



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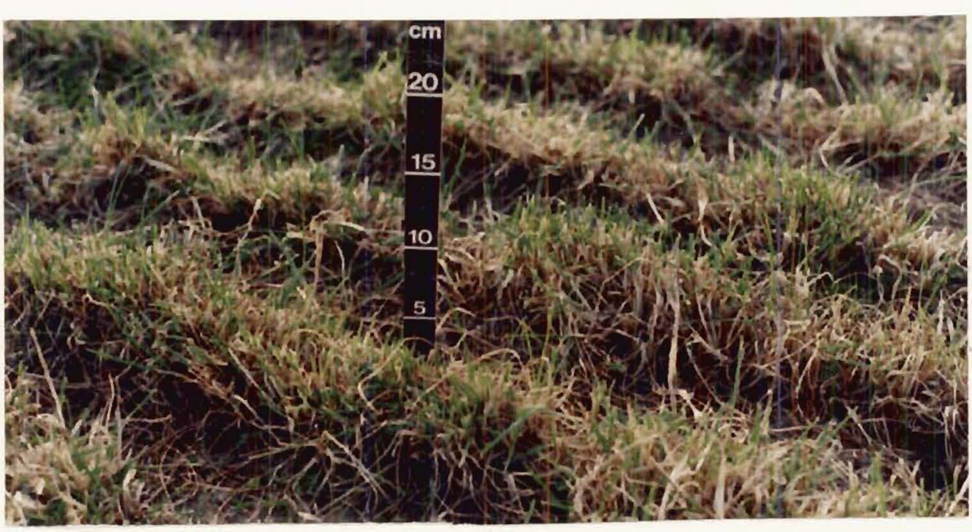

c

Plate 2 a,b,c Grass plots after 8 days of grazing in trial 1 (April 3-12) (a - tall fescue, b - +E ryegrass, c - -E ryegrass) throughout the grazing period to a maximum of 39% on day 6 of grazing (Figure 1).

When individual observation periods from the ten day duration of grazing are combined the proportion of total ewe hoggets grazing each grass was significantly higher for tall fescue than +E or -E ryegrass (Table 2). There was no significant difference between the proportion of total ewe hoggets grazing +E and -E ryegrass (Table 2).

Table 2 Proportion of total ewe hoggets grazing each grass over the ten day duration (April 3-12) of trial 1

Grass	Proportion of Total Ewe Hoggets Grazing Each Grass Treatment
tall fescue	0.38
+E ryegrass	0.32
-E ryegrass	0.29
l.s.d (5%)	0.04

5.1.3 GRASS HEIGHT

The grass height of +E ryegrass was significantly greater than -E ryegrass or tall fescue at start of trial 1 (Figure 2). This occurred in spite of pre-trial grazing and mowing techniques aimed at achieving the same height for each grass. The height of grass declined rapidly for all grasses over the first three days of grazing. As the duration of grazing proceeded past day 4 (6 April), the grass height of -E ryegrass and tall fescue continued to decline, while there was no change in the grass height of +E ryegrass which remained at about 65mm (Figure 2) (Plate 2 a,b,c). The height of grass was reduced 74%, 48% and 64% for tall fescue, +E ryegrass and -E ryegrass respectively over the grazing duration of ten days.

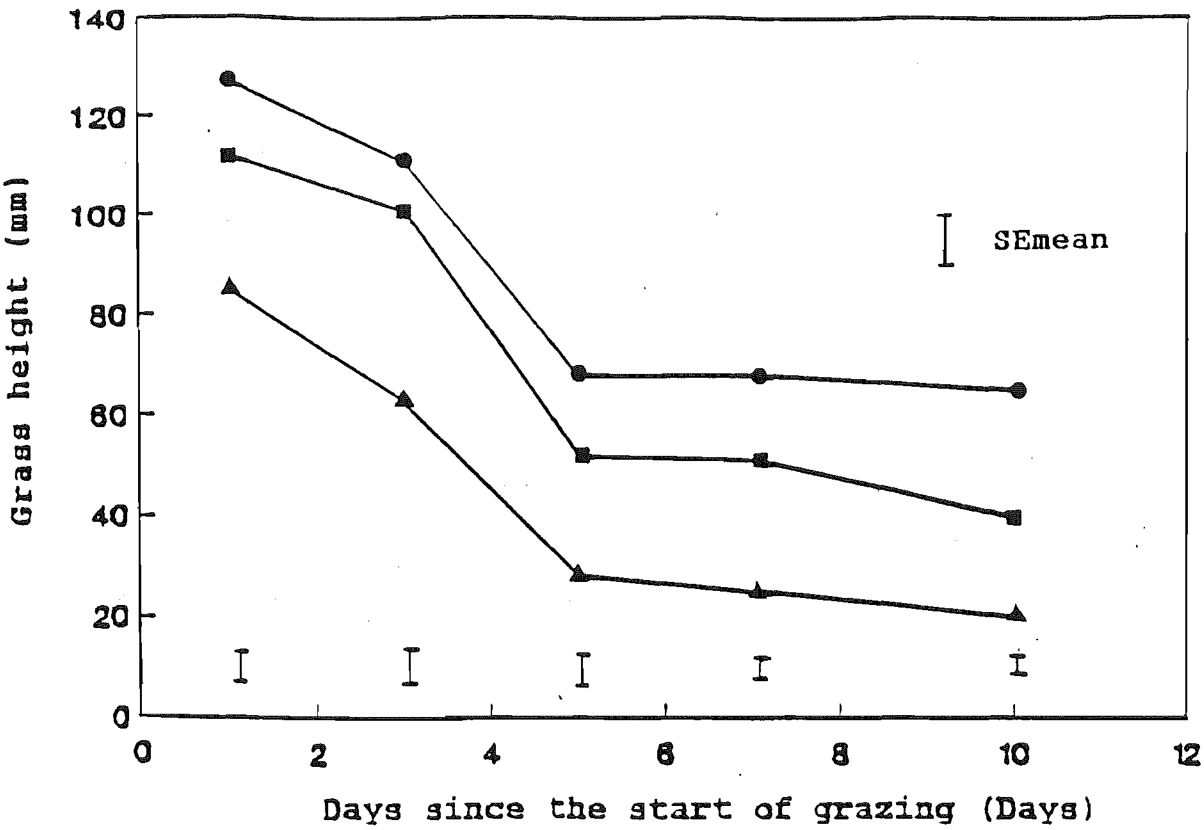

Figure 2 Grass heights during grazing in trial 1 (April 3-12). (▲) tall fescue, (●) +E ryegrass, (■) -E ryegrass

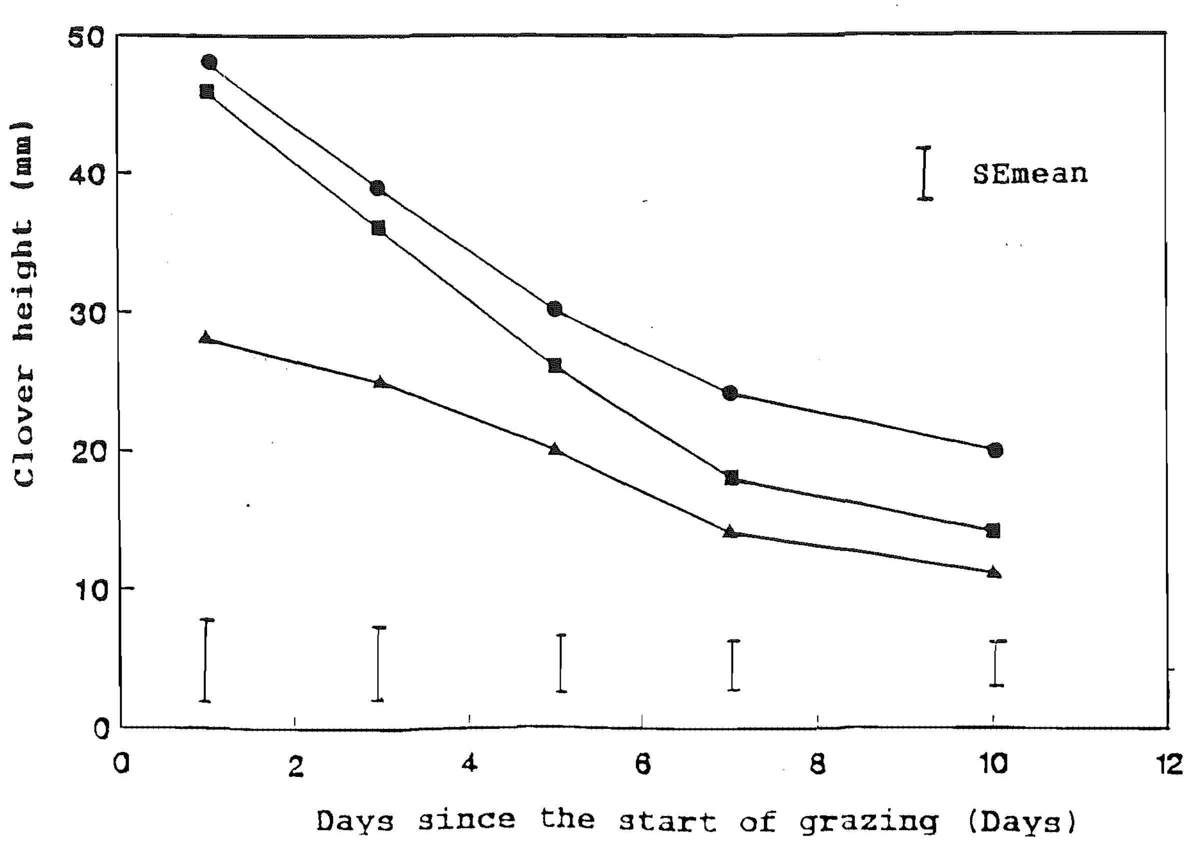

Figure 3 Clover height during grazing of grass/clover swards in trial 1 (April 3-12). (▲) tall fescue, (●) +E ryegrass, (■) -E ryegrass.

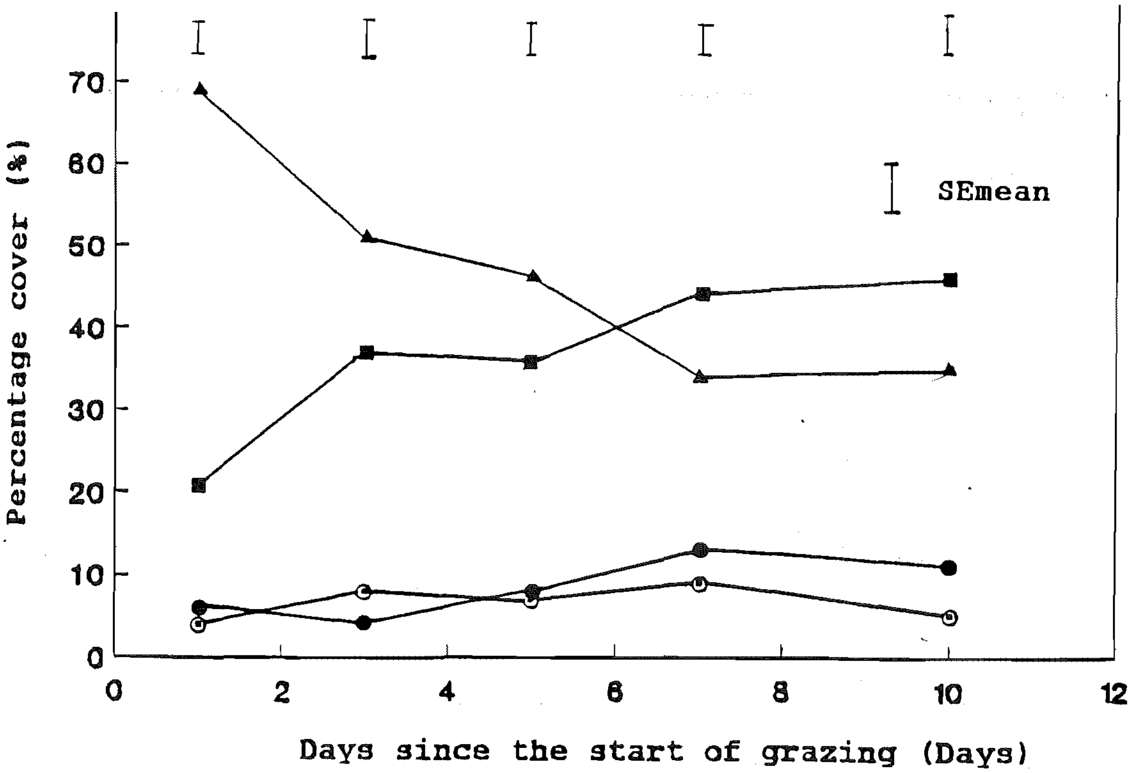

Figure 4c Percentage cover calculated from point analysis for -E ryegrass during grazing in trial 1 (April 3-12). (▲) green grass, (○) clover, (■) dead, (●) bare ground.

5.1.6 PASTURE COVER

Cover percentage of green grass, clover, dead and bare ground for tall fescue, +E ryegrass and -E ryegrass throughout the duration of grazing in trial 1 are shown in Figures 4 a,b,c. The cover percentage of four categories for +E and -E ryegrass were not significantly different prior to grazing. Tall fescue had a significantly higher percentage clover and bare ground and lower percentage dead than +E and -E ryegrass. Percentage green grass was similar between species. The percentage green grass decreased with grazing in all grasses but less in +E ryegrass (21%) than -E ryegrass (34%) and tall fescue (28%). Clover cover as low (4-7%) prior to grazing and generally declined with grazing. The percentage dead material by cover increased with grazing reaching highest levels in +E and -E ryegrass swards (46%). Percentage dead material by cover in ryegrass at conclusion of grazing was nearly twice that of tall fescue.

Bare ground on a percentage cover basis increased with grazing. Percentage bare ground cover for tall fescue (42%) at end of grazing was nearly four times that of +E and -E ryegrass.

5.2 TRIAL 2

5.2.1 PASTURE MASS

The pre-grazing pasture mass (kg DM/ha) of urine patches in tall fescue, +E ryegrass and -E ryegrass were 33%, 20% and 11.5% respectively greater than those of non-urine patches (Table 4). Post-grazing pasture mass (kg DM/ha) of urine patches were greater than those of non-urine patches but the difference was not as great as that for pre-grazing pasture mass except for +E ryegrass (Table 4). Urine patch post-grazing pasture mass was 24%, 27% and 3% greater than non-urine patch post-grazing pasture mass for tall fescue, +E ryegrass and -E ryegrass respectively.

Table 4 Pre-grazing and post-grazing pasture mass of grasses in trial 2 (May 14-21). (Urine and non-urine patch averaged over two mowing regimes.)

Grass	Pasture Mass (kg DM/ha)		
	Pre-graze	Post-graze	Change
Urine Patch			
tall fescue	1525	870	665
+E ryegrass	2415	1375	1040
-E ryegrass	2030	950	1080
Non-Urine Patch			
tall fescue	1015	660	355
+E ryegrass	1925	1000	925
-E ryegrass	1795	920	975

Pre-grazing pasture mass of +E ryegrass swards for both urine and non-urine patches were approximately 900 kg DM/ha higher than pre-grazing pasture mass of tall fescue swards. Pre-grazing herbage mass of -E ryegrass swards were intermediate between +E ryegrass and tall fescue (Table 4). Post-grazing pasture mass of +E ryegrass swards were higher than tall fescue or -E ryegrass swards.

Total pasture mass (kg DM/ha) of +E and -E ryegrass removed during the grazing period was far higher than that removed from

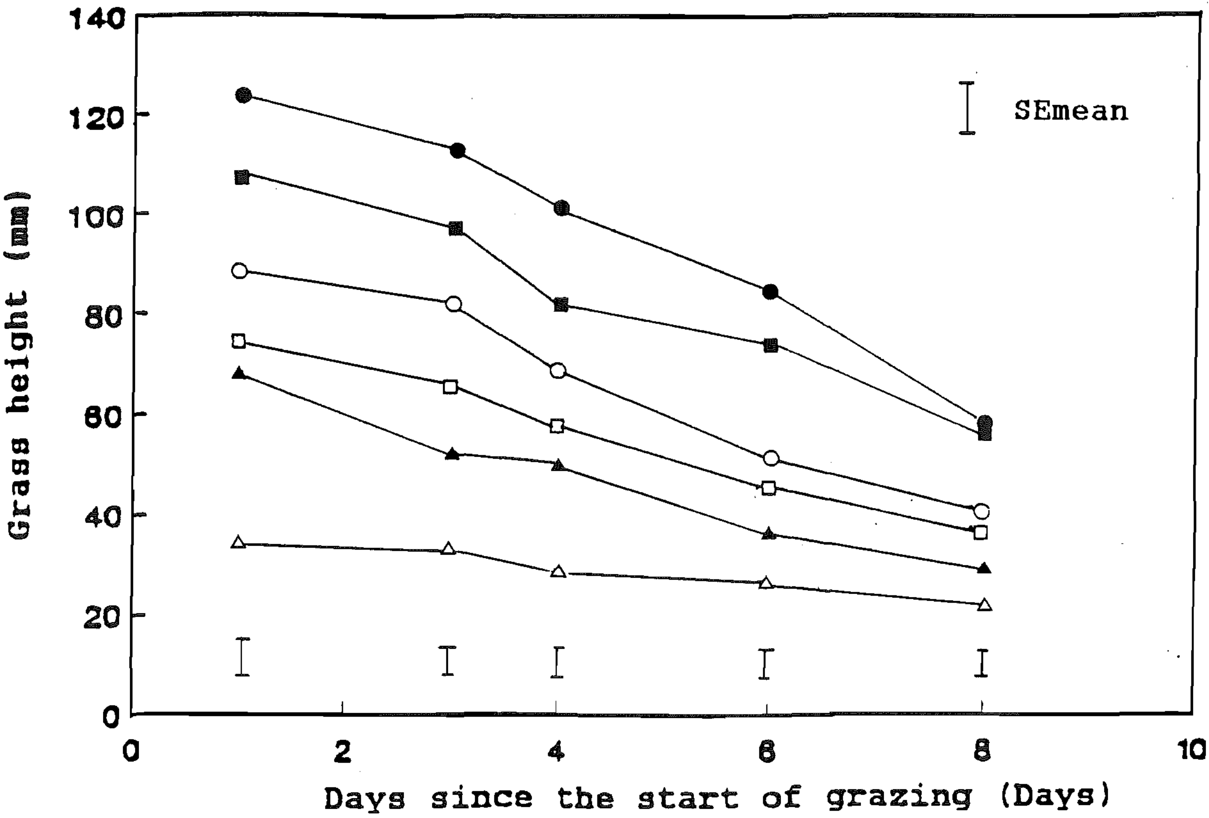

Figure 5 Grass heights in urine (closed symbol) and non-urine (open symbol) patches for each grass during grazing in trial 2 (May 14-21). (▲ △) tall fescue, (● ○) +E ryegrass, (■ □) -E ryegrass

tall fescue (Table 4). More pasture mass was removed from urine patches than non-urine patches for all grasses.

5.2.2 GRASS HEIGHT

The height on grass in mown plots was not significantly different from unmown plots. Therefore, results of unmown and mown have been averaged. The full set of results can be found in Appendix 1. The height of the +E ryegrass sward was significantly higher than -E ryegrass and tall fescue sward prior to grazing (Figure 4). The +E ryegrass swards were generally twice the height of tall fescue swards throughout grazing.

For all grasses the grass height declined with grazing, but at all stages of grazing urine patches were higher than non-urine patches (Figure 5). From day 1 to 3 of grazing grass height in urine patches declined more quickly that non-urine patches. Height of urine and non-urine patches declined at similar rates from day 3 onwards (Figure 5). Rate of height decline was not affected by mowing treatment.

At the conclusion of trial +E and -E ryegrass swards were of a similar height (Figure 4). These swards were twice the height of tall fescue swards at the conclusion of grazing.

5.2.3 PSEUDOSTEM HEIGHT

Mowing two weeks prior to grazing, produced a relatively constant pseudostem height (30-32mm) for urine and non-urine patches at the start of grazing (Table 5). Pseudostems, prior to grazing, were highest in unmown urine patch swards. The height of +E and -E ryegrass pseudostems prior to grazing were not significantly different but they were higher than tall fescue pseudostems in unmown plots.

Grazing reduced the height of pseudostems for all swards but this was only significant in tall fescue (Table 5). Mowing or urine did not significantly affect the amount of pseudostem removed.

Table 5 Pre-grazing and post-grazing pseudostem height of grasses in trial 2 (May 14-21) in plots that were either mown/unmown or urine/non-urine patch

Grass	Pseudostem Height (mm)			
	Urine Patch		Non-Urine Patch	
	Mown	Unmown	Mown	Unmown
Pre-graze				
tall fescue	30.0	32.0	31.0	32.5
+E Ryegrass	32.7	38.0	31.5	35.0
-E ryegrass	32.0	38.0	32.0	33.0
Post-graze				
tall fescue	21.3	26.3	25.0	27.5
+E ryegrass	31.0	37.0	30.0	35.0
-E ryegrass	31.0	37.0	32.0	31.0
l.s.d.(5%)	3.5			

5.2.4 PSEUDOSTEM HEIGHT IN TALL FESCUE/+E RYEGRASS ASSOCIATIONS

The pseudostem heights of +E ryegrass and tall fescue in pure and mixed associations are shown in Table 6. The height of tall fescue pseudostems were greater in mixed than pure clumps, while the height of +E ryegrass pseudostems were greater in pure than mixed clumps.

Table 6 Pseudostem height in pure tall fescue, pure +E ryegrass, and tall fescue ryegrass clumps (mm)

	Pseudostem Height (mm)
Pure tall fescue	19.5
Pure +E Ryegrass	35.8
tall fescue/+E ryegrass - +E Ryegrass	28.7
- tall Fescue	21.0
l.s.d.(5%)	2

Plate 3 Preferential grazing of tall fescue plots on day 1 of grazing in trial 2 (May 4-21)

5.2.5 OBSERVATIONS

No continuous observations were made in trial 2 similar to trial 1. However, it was observed that on the first day of grazing in trial 2 that most animals were grazing on tall fescue (Plate 3).

5.2.6 TILLER DENSITY

Tiller densities (number tiller/m^2) were greater in urine patches than non-urine patches for all three species (Table 7). Tiller densities were higher in +E ryegrass than -E ryegrass or tall fescue.

Table 7 Density of tillers (number/m^2) at thr start og trial 2 (May 14-21) urine and non-urine patches

Grass	Tiller Number/m^2
Tall Fescue	
Urine patch	5380
Non-urine patch	3475
+E ryegrass	
Urine patch	7407
Non-urine patch	4616
-E ryegrass	
Urine patch	6975
Non-urine patch	4592
SEmean	42

5.3 INDOOR FEEDING TRIAL

5.3.1 EXPERIMENT 1

5.3.1.1 Eating Rate

There was a significant effect of 'feed type' on the rate of eating of feed. This was evident on both a dry weight and wet weight (Table 8) basis. White clover was eaten faster than all other feeds on a wet and dry weight basis. Tall fescue was eaten quicker than +E or -E ryegrass on wet weight basis but only greater than +E ryegrass on dry weight basis. The -E ryegrass was eaten quicker than +E ryegrass on dry and wet weight basis.

Table 8 Effect of feed type on rate of eating on a wet (g WM/min) and dry (g DM/min) basis

Feed Type	Eating Rate	
	g WM/min	g DM/min
white clover	36.74	5.52
tall fescue	27.05	3.86
+E ryegrass	19.28	2.89
-E ryegrass	23.32	3.94
l.s.d.(5%)	2.41	0.619

The different rate of eating rankings between dry and fresh weight result from the different dry matter percentages of feed (Table 9).

Table 9 Dry matter percentages of feeds used in eating rate trial (Experiment 1 and 2)

Feed Type	Dry Matter %
white clover	15.0
+E ryegrass	15.0(a)
+E ryegrass leaf	15.4
+E ryegrass pseudostem	19.5
-E ryegrass	16.9(a)
-E ryegrass leaf	15.7
-E ryegrass pseudostem	21.9
tall fescue	14.3(a)
tall fescue leaf	14.5
tall fescue pseudostem	17.1

(a) Note: sampled at different times - refer methods

5.3.2 EXPERIMENT 2

5.3.2.1 Eating Rate

Table 10 shows the rate of eating (g WM/min and g SM/min) for the six feeds. On a wet and dry weight basis the rate of eating of the three leaf feeds (tall fescue leaf, +E ryegrass leaf, -E ryegrass leaf) were not significantly different, but the rate of eating of the three leaf feeds were significantly higher than the rate of eating of the corresponding pseudostem feed except for tall fescue on a dry weight basis (Table 10). Tall fescue pseudostems were eaten approximately two times quicker than +E ryegrass pseudostems on both dry weight and wet weight basis. The eating rate of -E ryegrass pseudostems was faster than that of +E ryegrass pseudostems (Table 10).

Table 10 Effect of different components of grass feeds on the rate of eating on a wet (g WM/min) and dry (g DM/min) basis

Feed Type	Eating Rate	
	g WM/min	g DM/min
+E ryegrass leaf	25.1	3.86
-E ryegrass leaf	26.8	4.21
tall fescue leaf	27.5	3.98
+E ryegrass pseudostem	9.5	1.85
-E ryegrass pseudostem	12.1	2.62
tall fescue pseudostem	20.3	3.47
l.s.d.(5%)	2.13	0.70

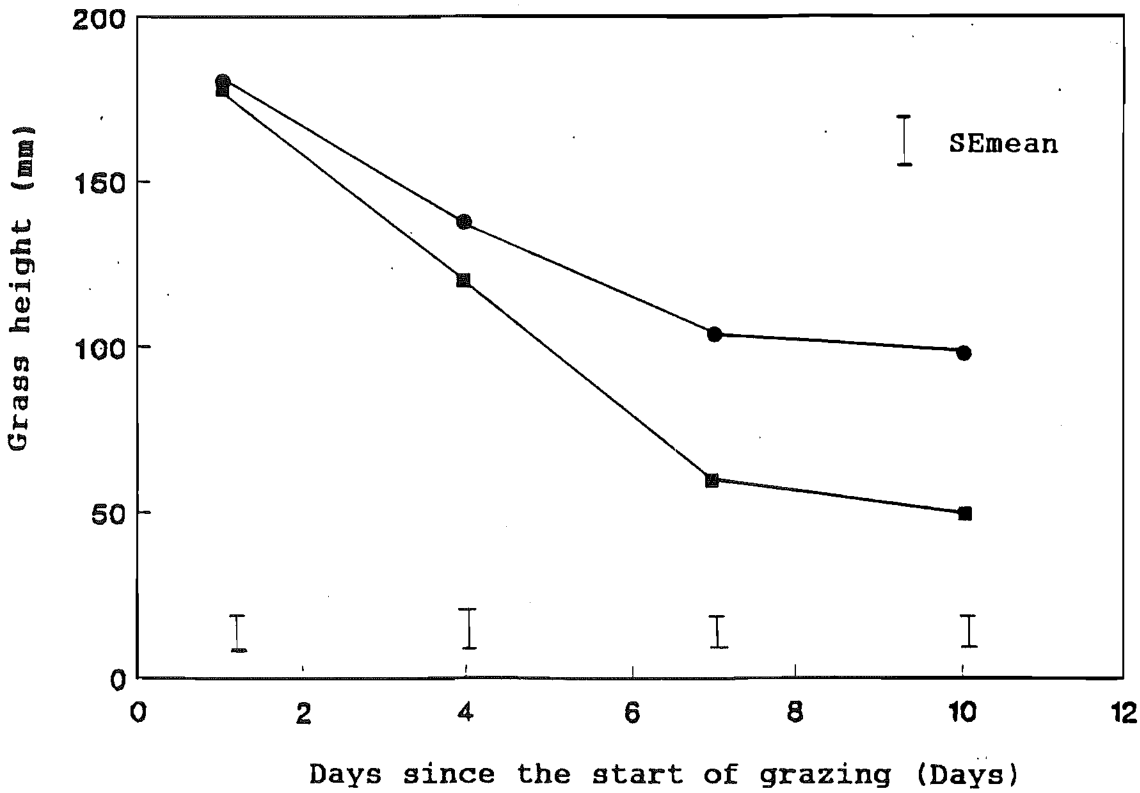

Figure 6 Grass heights during grazing in Ashley Dene trial (Spring 1990) (●) +E ryegrass, (■) -E ryegrass

5.3.2 EXPERIMENT 2

5.3.2.1 Eating Rate

Table 10 shows the rate of eating (g WM/min and g SM/min) for the six feeds. On a wet and dry weight basis the rate of eating of the three leaf feeds (tall fescue leaf, +E ryegrass leaf, -E ryegrass leaf) were not significantly different, but the rate of eating of the three leaf feeds were significantly higher than the rate of eating of the corresponding pseudostem feed except for tall fescue on a dry weight basis (Table 10). Tall fescue pseudostems were eaten approximately two times quicker than +E ryegrass pseudostems on both dry weight and wet weight basis. The eating rate of -E ryegrass pseudostems was faster than that of +E ryegrass pseudostems (Table 10).

Table 10 Effect of different components of grass feeds on the rate of eating on a wet (g WM/min) and dry (g DM/min) basis

Feed Type	Eating Rate	
	g WM/min	g DM/min
+E ryegrass leaf	25.1	3.86
-E ryegrass leaf	26.8	4.21
tall fescue leaf	27.5	3.98
+E ryegrass pseudostem	9.5	1.85
-E ryegrass pseudostem	12.1	2.62
tall fescue pseudostem	20.3	3.47
l.s.d.(5%)	2.13	0.70

5.4 ASHLEY DENE GRAZING TRIAL

5.4.1 GRASS HEIGHT

Grass height of +E and -E ryegrass was similar (180mm) at start of grazing (Figure 6). The grass height of the -E ryegrass declined quicker than the +E ryegrass (Figure 6). Total grass height reduction was 43% for +E ryegrass and 72% for -E ryegrass (Figure 6).

Distinct patch grazing occurred in +E ryegrass plots with clumps (1-2 m^2) of grass not being grazed.

5.5 ENDOPHYTE LEVELS

Table 11 shows the endophyte levels of ryegrass plants used in the feeding trial, and grazing trials (Lincoln and Ashley Dene). Endophyte levels in +E ryegrass in Trial 1 and 2 at Lincoln were low. No endophyte hyphae were detected in -E ryegrass at any site. Laxly grazed sites at Ashley Dene had higher endophyte incidence (70% v 30%) than hard grazed sites.

Table 11 Endophyte levels in ryegrass material for feeding trial and grazing trials

Grass	Endophyte Incidence % Infected Tillers
Feeding Trial	
+E ryegrass	70
-E ryegrass	0
Lincoln Grazing Trial 1	
+E ryegrass	40
-E ryegrass	0
Lincoln Grazing Trial 2	
+E ryegrass	0
-E ryegrass	0
Ashley Dene	
+E ryegrass	50
-E ryegrass	0
Ashley Dene	
+E ryegrass	
- lax graze	30
- hard graze	70

CHAPTER 6
DISCUSSION

6.1 SWARD CHARACTERISTICS BEFORE EXPERIMENTAL GRAZING

There were large differences in the characteristics of the grass swards prior to start of trial 1 and trial 2. It is important that these are discussed before interpretation of trial with reference to grazing preference takes place.

Endophyte levels in the +E ryegrass used for grazing trials 1 and 2 were not high. The percentage of infected tillers in +E ryegrass decreased from 40% at the start of trial 1 to 0% at start of trial 2 (Tablle 11). The mean level reported from a survey of 'Grasslands Nui' perennial ryegrass seedlines was 29% with a range of 10 to 85% (Thom et. al., 1987). The level of infection is dependent on parental source and storage process (Latch and Christensen, 1982).

The low levels in the experimental plots used in this study reflect the storage conditions and age of the seed. Seed stored at room temperature frequently loses its viability after two years while seed stored at low humidity and at temperatures below 0-5°C remains viable for up to 15 years (Latch, 1983). Seed, used in this trial, was over a year old and was not stored at low temperatures or humidity and therefore the viability of endophyte in the seed would be expected to decline. This would explain the low levels in trial 1, but levels declined from trial 1 to trial 2. This could be explained by the seasonal nature of endophyte mycelium growth. de Menna and Waller (1986) reported that the number of hyphae/mm breadth of leaf sheath at a site with a similar rainfall to Canterbury was highest in summer and lowest in winter. They proposed that the number of mycelia increased and decreased in parallel with temperature fluctuations. Therefore, the decline in endophyte infection could be attributed to the seasonal fluctuation in temperature.

While no quantitative assessment of the number of hyphae in infected tillers was made visual estimates indicated that there

Plate 4 a,b,c Sward structure at end of trial 1 (April 3-12) in grazed and caged areas of (a) tall fescue (b) +E ryegrass (c) -E ryegrass was extreme variability in infected tillers collected from each block. This observation is supported by di Menna and Waller (1986) who reported that the number of hyphae/mm breadth of leaf sheath varied by over ten-fold in infected tillers collected from the same site.

White clover contents of all swards were low, never exceeding 9% on a percentage cover basis (Figures 4 a,b,c, Plate 4 a,b,c). This was due to poor establishment of clover seed and the grazing management required to encourage the slower establishing tall fescue prior to the start of the grazing trial. Infrequent, lax, rotational grazing caused shading and allowed little light to penetrate to the base of the sward to initiate white clover stolons and leaves. No allelopathic effects of +E ryegrass on the level of white clover in the grass were noted. However, the low clover contents in all swards and the low endophyte levels make comparisons with reported literature (Sutherland and Hoglund, 1989) difficult.

There were also large differences between grass plots at the start of trial 1 in grass height, herbage mass, tiller density and amount of dead material.

The +E ryegrass tended to be of higher pasture mass, greater grass height and of higher tiller density than -E ryegrass and tall fescue (Figure 2, Table 1, Table 7, Plate 4 a,b,c). This occurred in spite of attempts to manipulate the swards to similar states by strategic cutting and grazing (Refer Section 4.1.2). An attempt to obtain a similar residual pasture mass over all swards four weeks prior to the start of grazing trial 1 by grazing with high stocking rates was largely unsuccessful due to the differential intensities of defoliation. During pre-trial grazing, sheep quickly defoliated tall fescue to a low pasture mass and then moved on to less preferred ryegrass species. However, sheep could not be left on plots for long enough to reduce herbage mass of ryegrass swards without damaging tall fescue swards through over grazing. Erection of temporary fences could have helped overcome this problem. In addition, there were different rates of recovery from pre trial grazing before trial 1 grazing.

The tall fescue plants were still relatively immature at six months of age when compared to the fast establishing ryegrass. The harder grazed tall fescue therefore had less green leaf area to help in recovery in comparison to ryegrass and hence recovery of ryegrass was more rapid.

There also appeared to be a difference in the orientation of tall fescue and ryegrass leaves. The regrowth of tall fescue leaves tended to be more prostrate than that of the ryegrasses. The sward height stick used would thus probably have underestimated the length of tall fescue leaves but would still have given the correct height of leaves above ground level.

Consequently, as a result of the lax defoliation of ryegrass relative to tall fescue in pre-trial management the +E and -E ryegrass contained a higher proportion of dead material than tall fescue.

Thus large differences existed in the state of the swards prior to the start of grazing. With the possibility that a multitude of factors could be influencing grazing preference, the difference in sward state makes it difficult to identify the major determinant of diet selection.

6.2 GRAZING PREFERENCE AND DIET SELECTION

6.2.1 INFLUENCE OF THE PSEUDOSTEM

Grass height declined rapidly in all three grasses over the first five days of grazing (Figure 2). From day 5 onwards the grass height of +E ryegrass remained constant while the grass height of -E ryegrass and tall fescue declined slowly (Figure 2). Although the sheep did penetrate more into the pseudostem horizon in -E ryegrass and tall fescue, the amount of pseudostem removed was small (Table 3). Sheep did not penetrate into the pseudostem horizon of +E ryegrass (Figure 2). This supports the suggestion of Barthram (1980) that while the pseudostem is not an impenetrable barrier it does clearly inhibit grazing. The ease of bite prehension is likely to be a major factor in the creation of the "pseudostem barrier". Arnold (1981) in his review of grazing behaviour stated that the range of grazing times for sheep was 4.5-14.5 hours with a mean of about 9 hours. It is conceivable in the process of evolution, that to avoid the onset of grazing fatigue from exerting varying amounts of force to harvest individual bites, an upper limit was set to the amount of force that could be exerted per bite. For such a mechanism to function the sheep would have to alter bite dimension so that the force exerted per bite stayed within the evolved range. The grazing animal may establish criteria as to the number of tillers that can be comfortably removed with each bite, and may determine that it is easier to prehend material above the pseudostem where the leaf is of smaller diameter, less fibrous and lower structural strength (Heady, 1975). Using these criteria the sheep will establish how deeply into the sward it will bite. This scenario would suggest that sheep can discriminate between plant components based on relative breaking strengths. Also it may explain why higher intakes are often achieved on tall leafy swards.

The stage of maturity and previous grazing management mean that pseudostems of different diameters and therefore breaking strengths can be found in pastures of a similar height. Consequently, the height of the pseudostem may influence the height to which a sheep prefers to graze. Where sheep are forced to graze pseudostems, pseudostem diameter is likely to influence bite depth and thus bite size. Pasture height alone without descriptions of other sward characteristics such as pseudostem height or diameter may therefore not always be an accurate determinant of bite size and intake.

The accumulation of pseudostems has important implications for grazing management. Parsons et. al. (1988) attributed some of the reduced efficiency of harvest occurring with a long duration of regrowth under rotational grazing to the accumulation of stems. In contrast to continuous grazing, severe defoliation under rotational grazing is not in itself effective in controlling the development of stems as these will accumulate if the duration of regrowth is sufficiently long. Not only may these stems be difficult to graze but they may also cause a reduction in total tiller numbers and thus prejudice the subsequent recovery of the pasture. This suggests that for rotational grazing the pasture should be grazed after the maximum average pasture growth rate occurs but before pseudostems accumulate and cause a marked deterioration in sward structure.

6.2.2 INFLUENCE OF ENDOPHYTE

The grass height of the three grasses during grazing would give an indication of how deeply the animals were penetrating into the sward, and possibly an indication of grazing preference. The +E ryegrass declined less (48%) in height than the tall fescue (74%) and -E ryegrass (64%) in trial 1 (Figure 2). A similar response occurred at Ashley Dene (Figure 6). The smaller decline of +E ryegrass was not due to its more vigorous growth and thus possible leaf extension rates as leaf extension rates were calculated from caged data and grass heights (Figures 2, 5 and 6) were corrected relative to these leaf extension rates.

The smaller decline in +E ryegrass grass height in trial 1 and the Ashley Dene trial (Figures 2 and 6) relative to -E ryegrass and tall fescue is supported by a number of other measurements made in this study. Firstly, green leaf cover declined less for +E ryegrass (21%) than -E ryegrass (34%) and tall fescue (28%) (Figure 4 a,b,c). Secondly, the amount of +E ryegrass pasture removed during grazing (1100 kg DM/ha) in trial 1 was intermediate between tall fescue (1020 kg DM/ha) and -E ryegrass (1240 kg DM/ha) (Table 1). Pasture mass removed from tall fescue was probably limited by pasture mass available as grazing proceeded. Thirdly, in the spring trial at Ashley Dene sheep grazed intensely in areas of +E ryegrass where endophyte incidence was low but laxly in areas where endophyte incidence was high. Finally in trial 2, where 0% endophyte infection of +E ryegrass was measured sheep ate as deep into +E ryegrass swards as -E ryegrass swards, and removed similar amounts of pasture mass (kg DM/ha). (Figure 5, Table 4).

Combining these results, there is a strong suggestion that sheep preferred not to eat the +E ryegrass when it was infected with endophyte. This appeared to be the situation even though more pasture mass, greater green leaf cover and greater leaf length was available in the +E ryegrass. Such apparent discrimination against +E ryegrass would be expected to be supported by the visual observations which recorded the number of sheep grazing on each plot.

However, while the visual observations of grazing in trial 1 show that initially more sheep grazed on tall fescue than -E or +E ryegrass, there was little difference in the number of sheep grazing each grass later in the grazing period (Figure 1). Total proportion of sheep grazing each grass over the whole duration of grazing was greater for tall fescue but similar for +E and -E ryegrass (Table 2). Consequently, it is surprising that if a similar number of sheep grazed +E and -E ryegrass, differences in the reduction of height, pasture mass and green leaf area between +E and -E ryegrass occurred.

The visual observation technique of observing animals from a tower 20-30 metres from each plot could have contributed to this phenomenom. Sheep were recorded as grazing if they were standing with head down and actually appeared to be grazing. However, due to attempts to observe sheep over the whole paddock in a short time period it is likely that sheep noted as grazing in fact were not. The photographic technique used by Hunt and Hay (1990) would have been a better alternative to study stock activity for an instant in time which could then be analysed at a later date. The main advantages of the photographic technique are rapid error free sampling, reliability and the ability to obtain preference sequences.

While the method used here and by Hunt and Hay (1990) to observe preferences both indicate which plot an animal is on and may provide information of grazing behaviour, neither allows the attributes of the sward just abandoned to be measured without disturbing grazing. Therefore, visual or photographic techniques must be supported by agronomic measurements on sward state, and even these will often be historical.

Thus the visual observation technique used could not accurately identify if sheep were actually prehending herbage. With indoor feeding trials using artificial swards or offered turfs this would be possible by recording head jerks associated with pasture removal. The total number of bites could be measured with a monometer (Black and Kenney, 1984).

Thus the agronomic measurements obtained suggest that sheep prefer not to graze +E ryegrass. If this is the case it is important to try to comprehend the mechanisms and reasons why the +E ryegrass is being avoided. The rate of eating trial gives greater understanding of this interaction and will now be discussed.

6.3 EATING RATE AND ITS RELATIONSHIP WITH GRAZING TRIALS

Intake rates ranged from 9 to 36 g WM/min and 1.85 to 5.52 g DM/min, but within each forage varied little between sheep (c.v. 14%) or between measurements with the same sheep (c.v. 8%) (Table 8 and 10).

Rates of eating on both a wet weight and dry weight basis are less than those reported for Italian ryegrass forages of different stages of maturity (Moseley and Antuna Manendex, 1989), dried forages (Kenney and Black, 1984) and from artificial kikuyu grass (Pennisetum clandestinum) swards. In the three cases above the eating rates obtained were for mature sheep which are likely to be heavier than the 25 kg sheep used in the present trial. Consequently, the larger animals were likely to have higher intake rates.

The method of measuring eating rate of forages was also different. Moseley and Antuna Manendex (1989) and Kenney and Black (1984) calculated eating rates by recording the amount of feed consumed in one minute intervals while this study calculated eating rates by recording the time taken to eat 100g of feed. Therefore, the slower eating rates observed may be related to the longer intake period. In addition forages were placed in large containers which may have restricted the recovery of herbage from the plastic base of the feed bin and slowed the rate of eating.

The dry matter percentages of the forages used in feeding trials ranged from 14.3 to 21.9% (Table 9). The dry matter percentages are similar to those reported for autumn pastures (Ullyatt et. al., 1980; John et. al., 1987). The dry matter content of pseudostem feed samples was higher than that of leaf feed samples. This could be attributed to the greater amount of dead material in pseudostem samples and increased lignification of pseudostem portions.

The dry matter percentages of unsorted material were determined at a different time to those of sorted material. This could explain the lower dry matter content of the unsorted +E ryegrass relative to +E ryegrass leaf (Table 9).

The -E ryegrass was of a higher dry matter content than +E ryegrass. This is surprising, considering feed samples were cut from plants at a similar stage of maturity and no visible differences in the proportion of dead material in feeds was evident. Dead material of feeds was not measured. On reflection, this would have been an important variable considering the influence it can have of diet selection in the field (Geenty, 1983).

Black and Kenney (1984) suggested that there was deliberate preference for foods that could be eaten faster. However, in this trial no preference tests (i.e. choice tests) were performed to see if a similar relationship existed. Unsorted tall fescue and white clover were eaten faster than the two ryegrasses (Table 8). This may reflect the smaller particle size of tall fescue and white clover which has been shown to increase intake rate and be preferred by animals (Black and Kenney, 1984). In the field sheep spent more time grazing tall fescue initially than +E or -E ryegrass (Figure 1, Plate 3). This may reflect an initial preference for tall fescue when it is freely available. Later in grazing the reduced pasture mass of tall fescue would restrict intake and the sheep may move onto swards where more pasture was available. If sheep can eat white clover quickly it may be preferred. Consequently, the greater amount of sheep grazing tall fescue early in grazing may be taken to represent preference for the more accessible white clover leaf in the more prostrate tall fescue sward. However, this was not supported by rapid declines in clover height or clover percentage over this time relative to +E and -E ryegrass (Figure 3; 4 a,b,c).

For all three grass feeds pseudostems were eaten slower than leaves. However, the difference in the rate of eating was more pronounced in the ryegrass feeds than the tall fescue. The sheep still ate the tall fescue pseudostems at a high rate (Table 10). In general, pseudostem material was shorter than leaf material so if particle length was to have any influence on eating rate it would be in favour of pseudostems due to fewer manipulative and chewing bites being required (Kenney and Black, 1984). Pseudostem feed samples contained more dead material, and have greater structural strength (Heady, 1975) than leaf feed samples. This would increase the time required to manipulate and chew feeds and thus slow eating rates. Pseudostems are also likely to be more harsh to touch and may be rejected by animals. The slower eating rate of pseudostems supports the concept of a pseudostem barrier in grazed swards.

The +E ryegrass was eaten slower than the -E ryegrass (Table 8). Feed intake has been shown to be depressed and animal performance reduced by the presence of endophyte and accompanying toxins in fescue (Jackson et. al., 1984, Goetsch et. al., 1989). The latter authors found that total dry matter intake by steers as a percentage of body weight decreased linearly by 0.0055% for each 1% increase in dietry endophyte infected tall fescue hay offered. Intakes of diets high in endophyte were lower than could be explained by ruminal-fill factors.

Stage of maturity and dead material content of +E and -E ryegrass feeds appeared similar and therefore should not have influenced eating rate. However, -E ryegrass had a higher dry matter content than +E ryegrass (16.9 v 15%). Interpreting this in view of the findings of Kenney et. al (1984) who found that the potential dry matter intake rate of kikuyu grass (Pennisetum clandestinum) by sheep increased as dry matter content increased up to a maximum of 40%, and John and Ulyatt (1987) who found that voluntary dry matter intake of ryegrass and tall fescue cultivars by sheep was positively correlated with dry matter content we may hypothesize that dry matter intake of -E ryegrass was higher than +E ryegrass due to its greater dry matter content. However, in the study of Kenney et. al. (1984) increases in dry matter content also reduced potential wet matter intake rate. Such reductions in wet matter intake at the higher dry matter content of -E ryegrass were not evident. Additionally while tall fescue and white clover had lower dry matter contents than -E ryegrass, both the rate of dry and wet matter intake were equal to or greater than -E ryegrass (Table 8). Thus inconsistent responses to dry matter content make it difficult to attribute differences in rate of eating to dry matter content of feed.

Feed samples of both +E and -E ryegrass were cut from pure swards of similar grass leaf height (120mm), and pseudostem height (35mm). Therefore, the quicker eating rate cannot be attributed to differences in the number of manipulative and chewing bites required for grass material of different lengths (Kenney et. al., 1984).

Examination of eating rates of separated pseudostem and leaf components indicates the reason for the slower eating rate for unsorted +E ryegrass.

When pseudostem and leaf components are offered separately there is no difference in the rate of eating of +E and -E ryegrass leaves but -E ryegrass pseudostems are eaten significantly faster than +E ryegrass pseudostems. This would suggest that if an acceptability factor was influencing the preference for a feed that it was being detected in the pseudostem component of +E ryegrass.

Returning to the results of grazing trial 1 it was noted that sheep were reluctant to penetrate into the pseudostem horizon of +E ryegrass and consequently grass height remained above that of -E ryegrass and tall fescue. This supports the slow eating rate of +E ryegrass pseudostems. Pseudostems contain the highest concentration of A. lolii mycelium in vegetative tillers (Keogh, 1986). Consequently, it is possible that sheep can detect the endophyte mycelium in the pseudostem and thus prefer not to eat it. Thus slower eating rates of +E ryegrass pseudostem and smaller declines in grass height of +E ryegrass occur.

Results from the patch grazing at Ashley Dene suggest that the sheep detected something in the upper horizons of sward as clumps were left completely ungrazed. The tillers at Ashley Dene would have been reproductive as the pasture was last grazed in May 1990. The highest concentration of A. lolii will be in the leaf sheath but the fungus is also present in the reproductive tiller albeit in lower concentrations (Musgrave and Fletcher, 1984). Therefore, the feeding deterrent could still be A. lolii hyphae. Farmers commonly claim that pasture that has begun to go reproductive is avoided by grazing sheep. This is particularly noticeable in pastures that have been spelled for long periods over the winter. The avoidance is usually attributed to the discrimination against the greater structural strength and size of reproductive tillers. However, the fact that reproductive tillers contain endophyte suggests that avoidance of endophyte may also be involved. Therefore an investigation examining the distribution and quantity of endophyte hyphae in reproductive tillers at different stages of maturity is of importance. Endophyte in seed could contribute to the observation that animals dislike eating ryegrass seedheads.

However, the patch grazing at Ashley Dene where animals left whole clumps ungrazed also raises the possibility that something being translocated through the plant is being detected.

The A. lolii - perennial ryegrass association produces the neurotoxins lolitrem A and B (Gallagher et. al., 1981) and the feeding deterrent peramine (Gaynor and Rowan, 1985). These compounds are involved with animal health and insect resistance. Is it not possible that peramine an appetite inhibitor of Argentine stem weevil (Gaynor and Rowan, 1985) is influencing the appetite of animals in the same way. Lolitrem A and B may have similar appetite inhibition properties. Studies of the distribution of these compounds throughout the plant and the influence that these have on livestock intake may be beneficial in the future. But the question remains, how is the presence of endophyte or chemicals being detected?

Brobeck (1957) suggested that intake facilitation or inhibition responses to food on offer in ruminants were associated with neural pathways responsible to visual, olfactory, auditory, tactile and gustatory stimulation.

Of the senses taste and smell are the likely receptor systems. Sight and touch are not likely to play a part as -E ryegrass and +E ryegrass look similar and have similar physical characteristics. Internal factors in detection following the digestion of +E ryegrass are unlikely because intake was depressed immediately in the indoor feeding trial. Whether long term reductions in intake similar to those observed by Goetsch et. al. (1987) for endophyte infected tall fescue occur in +E ryegrass is not known.

If the sheep can sense some factor associated with +E ryegrass this may explain why visual observations of the sheep did not detect any difference in the number of sheep grazing +E and -E ryegrass in trial 1. Only a proportion of tillers are infected with A. lolii (Section 6.1). Therefore, the sheep may have been moving over the +E ryegrass smelling of sampling sward and only pre-hending tillers that had low endophyte incidence. This situation existed in the spring trial at Ashley Dene where sheep grazed intensely in areas of +E ryegrass of low endophyte incidence but more laxly in areas of high endophyte incidence. Subsequently, the sheep may spend a long time on +E ryegrass without prehending large quantities of grass.

The apparent conflict between the time spent grazing the +E ryegrass and the amount of material removed from -E ryegrass could also be explained by the possibility of time consuming selection for white clover within the +E ryegrass sward.

Diet selection for white clover has been reported by some workers (Clark et. al., 1982; Bootsma et. al., 1980) but not others (L'Huillier et. al., 1984). The extremely low white clover contents of all grasses makes prediction that white clover was harvested more strongly in one grass difficult. The height of white clover in tall fescue was less than that of +E or -E ryegrass. This reflected the height of the grass sward. Petiole lengths were extended in the tall swards in an attempt to overcome shading. White clover height declined more quickly in +E and -E ryegrass but never reached the height of white clover in tall fescue. White clover plants in tall fescue had characteristics of more severe defoliation - small leaves and many small stolons, at the conclusion of grazing in trial 1. In the tall fescue the greater pseudostem free space between drill rows due to the lower tiller density would have allowed the sheeps mouth to penetrate closer to the ground and graze white clover more intensively.

The height of both +E and -E ryegrass were significantly higher than tall fescue at the start of trial 1 and 2. Tiller density was greatest for +E ryegrass (Table 7). Clark et. al. (unpublished) reported that sheep preferred grazing tall swards when offered swards of differing height, while Black and Kenney (1984) found sheep generally prefer long swards to short swards. But sheep at the start of trial 1 and trial 2 showed a preference for the shorter tall fescue swards. It is possible that sheep were discriminating against tall dense swards evident in the +E and -E ryegrass, and eating the short tall fescue because it could be eaten faster than +E or -E ryegrass. The natural correlation between height and density in pasture makes it difficult to manipulate one without influencing the other and therefore in many experiments confounding results are obtained.

It is possible that it was not an avoidance of tall pastures per se but avoidance of some other factor. The +E and -E ryegrass in trial 1 contained more dead material than tall fescue. This was due to the fact that tall fescue was grazed to a lower residual in the pre-trial grazing. Therefore, the tall fescue sward had younger leaves and contained less dead material. Animals consistently select more green than dead material compared to the sward from which it is harvested (Geenty, 1983). Therefore, the initial preference for tall fescue in trial 1 may be selection for a sward of lower dead material content. However, it was noted that most of the dead material was in the lower horizons for all grasses. Therefore, it is unlikely that dead material would influence grazing preference in the initial stages of grazing. Later in the trial when sheep were grazing the lower horizons, the lower herbage mass of the tall fescue would have meant that in order to maintain intake the sheep would have had to graze +E and -E ryegrass regardless of dead material content.

6.4 GRAZING RESPONSE TO URINE PATCHES

Keogh (1986) reported that grazed pastures are not uniformly defoliated by livestock during summer and autumn. Urine patch sites are grazed more frequently and intensively during those seasons.

The results of trial 2 indicate that urine patches were defoliated slightly quicker than non-urine patches so that the height difference that existed in all grasses at the start of grazing was reduced by the end of grazing (Figure 5). In addition, more pasture mass was removed from the urine patches (Table 4).

No endophytic fungi were detected in the +E ryegrass in May during trial 2 grazing. Keogh (1986) noted that the development of A. lolii is stimulated by nitrogen with comparative yields of endophyte mycelium being increased four-fold in urine patch sites. Endophyte levels in this study were measured over the whole of the ryegrass plots and no attempt was made to differentiate between urine and non-urine patches.

The finding of Keogh (1986) that sheep defoliate urine patches more intensively than non-urine patches conflicts with the results obtained in trial 1 and the eating rate trial. If the urine patches have a higher incidence of endophyte mycelium avoidance of these areas would be expected. However, some other overriding factor may make urine patches more acceptable to animals. This may include the chemical status of nitrogen, phosphorus or potassium.

If fungal concentration is greater in urine patches the close grazing of these sites could contribute disportionately to the acquisition of fungal toxins by livestock.

Mowing of plots created a constant pseudostem height (30-32mm) but this did not appear to have any affect on the rate at which grass height declined or the amount of pasture mass removed.

CHAPTER 7
GENERAL DISCUSSION

7.1 ALTERNATIVES TO ENDOPHYTE INFECTED RYEGRASS

In pastures dominated by +E ryegrass sheep dislike eating endophyte infected ryegrass and consequently animal performance would be expected to be lower. If this is the case farmers have the option of sowing nil endophyte ryegrass. However, a decision to do so must be seriously considered. Basically, the question breaks down to deciding whether to sow -E ryegrass which has low persistence in the presence of Argentine stem weevil (ASW) but is preferred by stock and likely to have higher clover contents - or to sow +E ryegrass which has high persistence but reduced clover content and causes animal health and performance problems.

On farms of relatively high rainfall or on irrigated farms where drought stress and overgrazing of pastures is less likely -E ryegrasses have a better chance of surviving summer attacks of ASW. Nevertheless ASW is still likely to cause tiller deaths in areas where ASW is active and animal production per hectare may be reduced. However, in cool moist areas (e.g. Southland) there is little reason for the farmers to sow +E ryegrass.

A possibility to overcome animal health problems is to sow +E ryegrass of varying levels of endophyte incidence. For example, 20% endophyte incidence may still give high persistence but cause less animal performance and health problems. But, trial 1 indicates that sheep disliked eating +E ryegrass when the endophyte infection level was 40% which is low relative to many of the high endophyte grasses now available (Yatsyn, Droughtmaster). In addition, the greater competitive ability of +E ryegrass is likely to mean that the level in pasture would increase.

The most recent research work has been towards the development of the 'novel' endophyte. 'Grasslands Pacific' a new perennial ryegrass that contains 'novel' endophyte has been bred from crosses between New Zealand, Spanish and Italian material. The endophyte strain in this ryegrass still offers protection against ASW but does not produce the lolitrem toxin which is believed to cause staggers (R.J.M. Hays, pers.comm.). Production of 'Grasslands Pacific' was equal to or greater than that of Nui, Yatsyn 1 and Ellet at Gore, Normanby and Palmerston North but only as good as Nui and Yatsyn 1 at the single dryland site - Lincoln (Pennell, et. al., 1991). This suggests that persistence of 'Pacific' may be lower in dryland environment. Field grazing experiments on 'Grasslands Pacific' are currently (Summer 1990/91) being done by L.R. Fletcher at Lincoln and will indicate whether or not field performance meets expectations.

'Grasslands Pacific' may have enhanced persistence with resistance to ASW over zero endophyte ryegrass but the exact relationship between the endophyte fungus and its detrimental effect on animal performance, animal intake and white clover content of sward have yet to be determined. An understanding of the mechanisms involved in fungus-animal interactions and possible toxins involved in these processes is imperative to the future use of novel endophytes.

The avoidance of +E ryegrass is correct in an evolutionary sense with respect to the fungus. The only means of dissemination of A. lolii is by seed (Seigel, et. al., 1987). Therefore, it would be advantageous to the fungus for the infected ryegrass plant to produce reproductive structures so that the fungus could be spread with the seed. Thus we may hypothesize that the avoidance by animals of +E ryegrass when offered is an adaptive mechanism of the fungus to ensure its continued survival and spread.

7.2 PREFERENCE

Preference is a rather nebulous term depending on a host of animal and plant factors. Additionally, opportunity and accessibility are purely subjective undefined terms. Preference has a time scale when choice between plant species is examined in the field. Unless material of the same nutritive value is being replaced in the same strata as that of each prehension bite at the same rate as consumption, the relative preference for remaining material changes. This situation is demonstrated by analysis of the changes in grass height during grazing in trial 1. Over the first two days of grazing height declined faster in tall fescue swards than ryegrass swards. This is supported by the initial observation results which showed that sheep spent more time grazing tall fescue swards in this time period. However, the greater initial removal of green leaf without replacement would markedly change sward characteristics. Consequently from day two to four of grazing, more leaf was removed from and more sheep grazed on ryegrass swards. It may be assummed from this that once sufficient of the preferred 'species' is removed the sheep will then move on to the less preferred species. This suggests that preference must be defined at a single point in time as preferred grazing behaviour markedly alters sward characteristics. Sheep may in a mixed sward remove the accessible components that they prefer but once the opportunity for selection of these components has been removed or 'barriers' to selection exist preference and diet selected may alter dramatically.

The visual observation technique used to record grazing preferences is limited because continuous records were not possible. Also it did not allow the attributes of the 'species' just abandoned to be measured and described without disturbing grazing.

CHAPTER 8
CONCLUSION

Large differences existed in the characteristics of the swards at the start of grazing trial 1. The +E ryegrass was of greater pasture mass, greater grass height and higher tiller density than -E ryegrass or tall fescue. The +E and -E ryegrass also contained a higher proportion of dead material than tall fescue. The large differences in sward characteristics make interpretation of results with respect to grazing preference very difficult.

The endophyte levels in +E ryegrass in the autumn grazing trials were low reflecting the seed storage time and conditions, and the seasonal nature of endophyte growth.

Sheep appeared reluctant to penetrate into the horizon containing pseudostem in all grasses. The ease of bite prehension is likely to be a major factor in the creation of the 'pseudostem barrier'. This 'pseudostem barrier' could have a serious effect on animal intake. Pseudostems of different height and diameter will exist in pasture of the same height depending on previous management and this should be considered when grazing management strategies are being devised.

When endophyte infected tillers were present in +E ryegrass sheep did not graze as deeply into the sward as in -E ryegrass. This occurred in both spring and autumn. Less pasture mass and green leaf were also removed from endophyte infected +E ryegrass than -E ryegrass. Combined with the results from the eating rate experiment which show that +E ryegrass was eaten slower than -E ryegrass primarily due to the slower eating rate of +E ryegrass pseudostems it is suggested that sheep prefer -E ryegrass to +E ryegrass. The sheep are likely to be sensing (taste/smell) either endophyte mycelium or chemical products produced by the endophyte-ryegrass association. It is proposed that the poorer animal performance reported, when animals graze +E ryegrass, is associated with reduced intake of +E ryegrass pasture.

It is also hypothesized that the prevention of sheep grazing +E ryegrass by the fungus is an adaptive mechanism of the fungus to allow continued spread and survival via seed transmission.

Urine patches were defoliated slightly quicker than non-urine patches. If urine patches contain a higher proportion of endophyte than non-urine patches the result of this defoliation pattern seems contrary to the earlier findings with respect to avoidance of +E ryegrass.

From the results of this study future research is suggested in the following areas:

1. Identify mechanisms by which consumption of infected ryegrass reduces intake and determine modes of preventing this depression. This would include analysis of the distribution and quantities of endophyte hyphae and chemicals produced by A. lolii-ryegrass associations throughout both vegetative and the reproductive tillers in all seasons of year.

2. Grazing preference studies on +E/-E ryegrass similar to trial described here but without the confounding influence of another species. Trials would be required to run in all seasons of year to determine whether the seasonality of endophyte levels influences preferences.

3. Grazing trials on +E/-E ryegrass throughout the year where animal performance is recorded.

4. Grazing studies on the pattern of defoliation of urine and non-urine patches in the sward ensuring that endophyte levels and chemical status at the two sites are measured. This may include observations of the defoliation pattern of marked tillers.

5. Indoor feeding trials examining the influence of endophyte infected ryegrass on short term and voluntary intake. The effect of increasing the proportion of +E ryegrass in the offered diet should be examined.

6. Choice tests to determine relative preference for +E or -E ryegrass. These could be performed by offering artificially prepared swards or cut turfs to animals.

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Appendix 1 Height of grass leaf in urine (+N) and non-urine (-N) patches under two mowing regimes during the grazing period of trial 2 (May 14-21)

Days Since Start of Grazing	Grass Height (mm)											
	Mown 2.5cm						Unmown					
	tall fescue		+E ryegrass		-E ryegrass		tall fescue		+E ryegrass		-E ryegrass	
	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N
0	68	38	124	82	99	71	68	44	129	92	116	78
2	55	37	110	80	96	69	49	29	116	89	100	69
3	53	29	97	75	80	54	48	27	104	63	84	63
5	35	29	78	50	68	46	38	28	93	59	80	54
7	28	25	50	47	57	42	31	24	62	58	58	43