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**AN EVALUATION OF THE NUTRITIVE VALUE
AND ENDOPHYTE STATUS OF A NEW
PERENNIAL RYEGRASS (*Lolium perenne*)
CULTIVAR (ARIES HD)**

A thesis presented in partial fulfilment
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ABSTRACT

Four grazing field experiments were carried out at Massey University, Palmerston North, New Zealand, to evaluate the nutritive value and endophyte status of a new perennial ryegrass (*Lolium perenne*) cultivar (Aries HD) selected for increased organic matter digestibility in summer and early autumn. It is claimed to be the first commercial perennial ryegrass selected specifically for improved digestibility. The nutritive value and organic matter digestibility of Aries HD, compared to a standard cultivar Yatsyn 1 perennial ryegrass were evaluated in terms of liveweight gain, carcass weight gain, wool production, grazing behaviour and herbage intake of sheep. The effects of endophyte (*Neotyphodium lolii*) were assessed with reference to the performance of sheep, incidence and severity of ryegrass staggers, serum prolactin concentration, respiration rate, rectal temperature, faecal moisture and scouring (dags). A tiller demography experiment was carried out to compare the survival, reproductive development and density of tillers in pure swards of Aries HD and Yatsyn 1 pasture.

The first three grazing experiments (Experiments 1, 2 and 3) were conducted on clover-free swards of Aries HD and Yatsyn 1 established in the autumn of 1995, in a randomised complete block design with three blocks (0.33 ha/plot). The proportion of tillers infected with endophyte was over 90% in both cultivars. Plots were continuously grazed to a sward surface height of 6 cm. There were regular monthly applications of nitrogenous fertiliser and herbicide was applied to eliminate volunteer clover. *In vitro* organic matter digestibility, neutral detergent fibre and nitrogen content from herbage cut to ground level or plucked samples did not differ significantly overall, and showed no indications of seasonal differences between cultivars.

In the first weaned lamb experiment (Experiment 1: 11 December 1995 to 25 April 1996) lambs grazing Aries HD gained 20 g/day more than lambs grazing Yatsyn 1 pasture (104 vs 84 ± 4.6 g/day, $P = 0.1028$), with a particular advantage in relative terms

over the dry summer period. This resulted in a 9% greater carcass weight at slaughter for Aries HD lambs over Yatsyn 1 lambs. Incidence of clinical ryegrass staggers among Yatsyn 1 lambs was double that of lambs grazing Aries HD (29 vs 15%) although lolitrem B concentrations did not differ between cultivars. Ergovaline concentrations in Aries HD herbage samples were consistently half those of Yatsyn 1 samples. The better animal performance in this experiment reflected the interrelated effects of alkaloid concentrations and ryegrass staggers.

Experiment 2 (3 September 1996 to 1 December 1996) measured the performance of ewes with their single lambs over spring, providing an evaluation of the relative nutritive value of pastures when the risk of endophyte alkaloids was minimal. Ewe liveweight gain was significantly higher on Aries HD than Yatsyn 1 over September (94 vs 56 ± 14.5 g/day) which coincided with a significantly higher bite rate and herbage intake. This enabled Aries HD ewes to gain an extra kilogram over the spring months. The liveweight gain of the suckling lambs did not differ between cultivars, presumably reflecting high and non-limiting milk yields on both cultivars. The percentage of leaf was consistently higher in Aries HD swards over this experiment, although this was not reflected in a higher organic matter digestibility.

In the second weaned lamb experiment (Experiment 3: 2 December 1996 to 12 March 1997) there was no significant difference in lamb liveweight gain (116 vs 111 ± 5.1 g/day) between cultivars. Incidence of ryegrass staggers was low with only 9% of Yatsyn 1 lambs being affected, which reflected low levels of lolitrem B and ergovaline. It was concluded that the lambs were faced with a lower alkaloid challenge than in the previous summer, reflected in the lack of difference in animal performance between cultivars.

The tiller demography experiment spanned both Experiment 2 and Experiment 3 (September 1996 to March 1997). Each replicate plot had five randomly placed transects with 10 marked tillers, and tiller survival and reproductive development were recorded at weekly intervals. Tiller population density and mean tiller weight were

determined on three dates. There was no difference in the rate of tiller death between Aries HD and Yatsyn 1. Few tillers died until mid December, after which survival approximated an exponential decay curve (e^{bt} , $b=-0.0133$, $t_{1/2}=52\text{days}$). Aries HD appeared to have a more rapid onset of initial flowering but then a lower proportion of secondary reproductive tillers. There was some evidence that the proportion of vegetative tillers was greater in Aries HD than in Yatsyn 1 swards. Aries HD swards had a higher density of finer tillers than Yatsyn 1.

The results from the first three experiments highlighted the need for more detailed evaluation of the cultivar/endophyte associations which influenced the production and balance of alkaloids. The final grazing experiment (Experiment 4: 2 December 1997 to 7 April 1998) was conducted with this in mind. Clover-free swards of Aries HD and Yatsyn 1 were established in the autumn of 1997 in six replicate plots (0.2 ha) of each cultivar arranged in a randomised block design. The proportion of tillers infected with endophyte was 96% in both cultivars. There were regular applications of nitrogenous fertiliser and herbicide was applied to eliminate volunteer clover and *Poa annua*. The experiment was designed as a 2 x 2 factorial, with two perennial ryegrass cultivars (Aries HD and Yatsyn 1) and two grazing sequences. Two groups of lambs were rotationally grazed on each cultivar in a leader/follower sequence. It was anticipated that the leader lambs would test the nutritional value of the pasture, while the follower lambs would be forced to graze into the base of the sward possessing the greatest potential for endophyte toxicity.

Lambs on all treatments were severely affected by ryegrass staggers from 3 February onwards. The leader-follower regime created contrasts in sward composition and nutritive value, resulting in significantly faster liveweight gains in leader lambs than in follower lambs (92 vs 53 ± 10.6 g/day). Aries HD and Yatsyn 1 pasture did not differ in *in vitro* organic matter digestibility, neutral detergent fibre, nitrogen content or in liveweight gain of lambs. Lambs grazing Aries HD pasture had higher herbage intakes in late January than those grazing Yatsyn 1 pastures. Ergovaline concentration of Aries HD pasture was consistently half that of Yatsyn 1 pasture. Respiration rate, which is an indicator of heat stress, was higher in Yatsyn 1 lambs. Staggers severity score was

highest in the Yatsyn 1 pastures. The higher ergovaline concentration may have acted synergistically with lolitrem B concentration to increase the severity of staggers observed in Yatsyn 1 pastures. The follower lambs had significantly reduced serum prolactin levels and respiration rates, possibly reflecting greater ergovaline intoxication, and had greater faecal contamination scores (dags) and severity scores. The severe and debilitating symptoms of ryegrass staggers could have prevented any differences in animal performance during the period when severe staggers were observed.

From this series of experiments it was concluded that the differences in lamb performance over summer and autumn reflected contrasts in the production and balance of endophyte alkaloids from the respective cultivar/endophyte associations. Small apparent differences in the reproductive development of tillers were not translated into any significant difference in the digestibility or nutritive value of the two cultivars, under either continuous or rotational grazing regimes. Aries HD in association with endophyte consistently produced half the concentration of the alkaloid ergovaline as did Yatsyn 1 in association with endophyte. Ergovaline may have acted synergistically to increase the toxicity of lolitrem B and the severity of staggers of lambs grazing Yatsyn 1 swards. There is also some indication that heat stress symptoms are more severe in lambs forced to graze lower into the sward.

This work highlighted the importance of assessing not only the nutritive value, but the effects of the cultivar/endophyte association in animal evaluations of perennial ryegrass in New Zealand. The effect of management and alkaloid concentration is likely to have a larger impact on lamb performance than small differences in nutritive value between cultivars of high-endophyte perennial ryegrass.

This thesis is dedicated to my parents Sue and Paul Bluett

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CHAPTER 1

GENERAL INTRODUCTION, OBJECTIVES AND FORMAT OF THE THESIS

1.1 GENERAL INTRODUCTION

New forage cultivars need to be evaluated using the grazing animal and under conditions in which they must perform (Morley 1978; Hodgson 1981; Ulyatt 1981a; Ceccarelli et al. 1992). Attempting to breed cultivars for improved nutritive value without conducting animal evaluation experiments could lead to false conclusions (Vogel and Sleper 1994). Careful testing of new ryegrass/endophyte associations is critical in identifying detrimental effects on the grazing animal (Siegal 1993). However, for financial and logistical reasons, few grazing experiments have been conducted where cultivar differences in nutritive value have been evaluated in terms of animal production (Evans 1979).

This thesis focuses on evaluation of the nutritive value and endophyte status of a new perennial ryegrass cultivar. Wrightson Seeds selected Aries HD perennial ryegrass for improved organic matter digestibility over summer and autumn (November to February). This encompasses the period when perennial ryegrass matures, which is associated with a decrease in apparent digestibility and nutritive value (Ulyatt 1981a and b). A small increase in organic matter digestibility could have a significant impact on animal production (Blaxter 1960; Corbett and Wheeler 1979; Vogel and Sleper 1994). Significant and large differences in lamb production have been demonstrated between cultivars of perennial ryegrass in the United Kingdom and have been related to differences in organic matter digestibility (Davies et al. 1989a, 1993; Evans et al. 1979). Wrightson Seeds have demonstrated an improvement of 2.6 % OMD over a standard cultivar, Yatsyn 1 perennial ryegrass, under intermittent cutting

management (Appendix 1.1). Liveweight gains of livestock can provide an overall assessment of forage quality and will show if selection for improved digestibility has resulted in improved animal performance (Vogel and Sleper 1994).

The majority of perennial ryegrass contains endophyte (*Neotyphodium lolii*; Glenn, Bacon & Hanlin = *Acremonium lolii*) which can affect animal performance over summer and autumn. Perennial ryegrass containing endophyte is now generally preferred by the pastoral industry in New Zealand because it is more persistent under threat from drought and pasture pests such as Argentine stem weevil (Fletcher and Piper 1990). Endophyte-infected perennial ryegrass can cause ryegrass staggers, reduced liveweight gains, increased faecal moisture (scouring) and faecal soiling (dags), increased incidence of flystrike, increased rectal temperatures and respiration rates, and depressed serum prolactin levels in grazing animals (Fletcher 1993a; Familton et al. 1995; Fletcher et al. 1996). The development of synthetic ryegrass/endophyte associations has the potential to manipulate alkaloid profiles and animal responses (Fletcher and Easton 1997).

The interaction between endophyte and ryegrass affects animal performance, therefore comparisons of perennial ryegrass in New Zealand must be interpreted with respect to their endophyte status. In the past the perception of genetic differences between cultivars in field performance has been impaired by the presence of endophyte (Easton 1983; Fletcher and Easton 1997). Both the host ryegrass cultivar and the strain of endophyte can determine the presence and quantity of alkaloids produced and animal response (Barker et al. 1993; Davies et al. 1993; Fletcher and Sutherland 1993a; Garthwaite et al. 1993; Powell et al. 1993b; Latch 1997). Concentrations of alkaloids can vary within different parts of the plant depending on the time of the year and the environmental constraints being imposed on the system (Familton et al. 1995). Tremorgenic alkaloids may act synergistically with other endophyte/ryegrass alkaloids in the incidence of staggers (Gallagher et al. 1977; Fletcher et al. 1993a; Clarke et al. 1996).

1.2 OBJECTIVES

This thesis reports the results of four field experiments in which particular attention was paid to:

- 1) Comparative evaluation of the nutritive value and organic matter digestibility of Aries HD and Yatsyn 1 perennial ryegrass and their effects on liveweight gain, carcass weight gain, wool production, grazing behaviour and herbage intake of sheep.
- 2) Concentrations of perennial ryegrass endophyte (*Neotyphodium lolii*) alkaloids, and their effects on the health and performance of sheep, incidence and severity of ryegrass staggers, serum prolactin concentration, respiration rate, rectal temperature, faecal moisture and scouring (dags).
- 3) Investigation of the relative patterns of survival, reproductive development, and density of tillers in pure swards of both ryegrass cultivars.

The series of experiments ran over three consecutive years to cover a range of environmental conditions, and under both continuous and rotational grazing management to maximise the opportunities to evaluate nutritional and endophyte related effects.

1.3 **FORMAT OF THE THESIS**

Chapter 2 is a review of literature reporting information on improving the nutritive value and digestibility of perennial ryegrass, evaluation of new cultivars and the effect of the *Neotyphodium lolii* endophyte on animal performance. Experimental material is presented as a series of chapters involving papers that have already been published (Chapter 3), are ready for submission to journals for publication (Chapters 4 and 5), or are being prepared for submission (Chapter 6 and 7). Each of these papers deals with a specific experiment, and an additional chapter (Chapter 6) reports the results of a set of sward studies which linked the experiments reported in Chapters 4 and 5. In all cases the paper presentation has been modified slightly to suit thesis format. A final chapter (Chapter 8) presents an integrating discussion and summary of all the experimental material.

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL INTRODUCTION

The objective of this thesis was to evaluate the nutritive value and endophyte status of a new perennial ryegrass cultivar (Aries HD) which was selected for improved organic matter digestibility over summer and autumn. The aim of this literature review is to investigate (1) breeding perennial ryegrass for improved nutritive value and digestibility; (2) the animal evaluation procedure for new cultivars; (3) the perennial ryegrass/endophyte association and its effect on animal performance.

The nutritive value and digestibility of perennial ryegrass declines as the plant matures over the summer, decreasing its feeding value. There is considerable variation within perennial ryegrass for characteristics associated with quality, which can be exploited by the plant breeder to improve animal production. Animal scientists and plant breeders have ranked increasing digestibility as the most important goal for improving milk production, liveweight gain and wool production (Wheeler and Corbett 1979, Smith et al. 1997). The potential pathways for improving digestibility and expected gains in herbage intake and animal performance are discussed. Perennial ryegrass cultivars selected for lateness of flowering, tetraploidy, shear strength and carbohydrate content are reviewed. The importance of animal evaluation trials in assessing new cultivars is emphasised. The design, management techniques, precision, cost and effectiveness of grazing experiments are investigated.

The ryegrass/endophyte association and its effect on the performance and health of grazing animals is reviewed. The alkaloids lolitrem B, ergovaline, paxilline and peramine are discussed in relation to their role in ryegrass staggers, heat stress, faecal contamination, and animal performance. The effect of the strain of endophyte and the

host ryegrass cultivar and the potential for manipulating alkaloid profiles is investigated. This literature review highlights the importance of evaluating both the nutritive value and the endophyte status of perennial ryegrass in animal trials.

2.2 BREEDING PERENNIAL RYEGRASS FOR IMPROVED NUTRITIVE VALUE AND DIGESTIBILITY

2.2.1 Perennial Ryegrass

Perennial ryegrass is the major component of New Zealand's high fertility pastures. The importance of ryegrasses has increased with the use of fertiliser so that they now account for over 90 per cent of pasture grass seed certified in New Zealand (Easton 1983). Ryegrass is a vigorously tillering, sward forming plant which originated in central Asia, around the Mediterranean and throughout northern Europe (Easton 1983). Thom et al. (1998) has reported cultivars available in New Zealand and their relative herbage production and persistence data. Some of the main benefits of ryegrass are its rapid establishment, good spring and autumn growth, excellent spring animal performance and its tolerance to a wide range of environmental and management conditions (Fraser 1997a). A disadvantage of perennial ryegrass is that it is difficult to manage to give high quality feed over summer, even with topping management (Fraser 1997a). Perennial ryegrass has limitations in nutritive value at some times of the year and is considered by some to be in need of improvement in this respect (Easton 1983; Reed 1987).

2.2.2 Ryegrass tiller demography and reproductive behaviour

In perennial ryegrass pasture the primary growth unit is the individual tiller and pasture can be described as a dynamic population of short-lived tillers. The life span of individual tillers varies from a few weeks to more than a year, so the continued production of new tillers that replace senescent or reproductive tillers ensures the perenniality of the plant (Hunt 1987). Rates of appearance, growth, and death of tillers in swards determine production, persistence and contribution to botanical composition

(Korte et al. 1985; Korte 1986). In summer, leafy vegetative swards are preferable to stemmy swards with a large proportion of dead herbage, because of their greater herbage production and quality (Korte 1984).

Tillers can die as a result of severe defoliation, shading, pulling, trampling, dung and urine deposition, winter-kill, competition for nutrients, disease and grazing. Reproductive tillers die when they are defoliated or when they reach maturity (Woodward 1998). Chapman et al. (1984) concluded that defoliation was the major factor contributing to the death of tillers throughout the year. Often the smallest vegetative tillers are the most vulnerable to death when the whole plant is stressed (Ong 1978a; Ong 1978b).

The main period of ryegrass tiller death is associated with reproductive development during November and December. Initially high tiller death rate reflects death of vegetative tillers, as reproductive tillers do not translocate assimilates to heavily shaded, smaller, vegetative tillers, and late from death of reproductive tillers severely defoliated as the shoot apex is elevated (Chapman et al. 1984; Korte 1986; L'Hullier 1987). Tillers that escape grazing at this stage can be grazed off after flowering, with some reproductive tillers escaping grazing altogether and dying in later summer at maturity (Chapman et al. 1984).

Tillers are induced to flower by low winter temperatures followed by increasing day length. About 6 weeks of cold (below about 10°C) are required to vernalise perennial ryegrass (Hunt 1987). Flowering results from a physiological change at the stem apex, which then produces a seed head but no further leaves, eventually resulting in the tiller dying (Hunt 1987). Woodward (1998) summarised the transition from vegetative to reproductive tillers (S24 ryegrass) as occurring in late winter, with stem elongation starting six weeks later in early spring, and ear emergence six weeks after that, by late spring. The first flowers form two weeks after ear emergence, and maturity follows a little over a month later, in early summer. The critical point in the reproductive development of a tiller is the onset of stem elongation, because the tiller can then be classed irreversibly as reproductive (Korte et al. 1984; Woodward 1998).

Korte et al. (1984) observed culm elongation in October and inflorescence emergence in November (50% inflorescence emergence on 23 November) in Nui perennial ryegrass rotationally grazed by sheep. Relatively few reproductive tillers appeared after December. Chapman et al. (1983) found about 75% of ryegrass tillers sampled in a hill country pasture showed evidence of reproductive development through stem elongation, but less than half of those were able to proceed to inflorescence emergence (mean date of inflorescence was 16 December). The rest were defoliated and had their elevating apex removed before this stage.

Tillering occurs in response to changing temperatures and light regimes, and is controlled by the rate of leaf appearance (L'Hullier 1987). Korte et al. (1985) identified two periods of rapid tillering in Grasslands Nui. The first was before culm elongation started (5-26 September) and the second after defoliation of the main group of reproductive tillers (7-28 November). L'Hullier (1987) observed tillering to be most rapid in ryegrass between November and January (later spring-early summer). Tiller density declined in spring and increased during autumn and winter.

Reduced tiller density has been associated with decreased net herbage accumulation rate (L'Hullier 1987) by restricting the number of growing leaves (Hunt 1987). Low tiller density can lead to poor persistence because of reduced resilience to further management stresses, and by increasing the risk of sward deterioration by factors such as pests, diseases, and physical damage. Reduced tiller density can also result in an increased occurrence of aerial tillering and sward pulling or winter-kill of tillers (L'Huillier 1987). Weaker genotypes could be crowded out by clumps of stronger genotypes should conditions allow it (Brock and Thomas 1991).

During the reproductive period (November to January) ryegrass plants are heavier and have more leaves and internode stolons than at other times of the year which is associated with flowering (Korte 1984; Brock and Thomas 1991; Brock and Fletcher 1993). Variation in tiller size has been observed between cultivars of perennial ryegrass and may be under genetic control (Brock and Fletcher 1993).

2.2.3 Definition of digestibility

Digestibility is the proportion of pasture consumed, which is digested (Ulyatt 1981a; Thompson and Poppi 1990). Digestibility defines the quantity of energy or nutrients available per unit of feed intake (Corbett 1978; Thompson and Poppi 1990). It is the summation of percentage content multiplied by percentage digestibility of all the different chemical components in the forage (Raymond 1969). Apparent digestibility can be expressed as follows:

$$\text{Apparent digestibility} = \frac{\text{Intake} - \text{Faecal output}}{\text{Intake}} \times 100$$

Digestibility depends on the proportion of cell contents that are completely digested and of the proportion of cell wall. The cell wall (cellulose and hemicellulose) is only partially digested, depending on the degree of lignification. The major cause of variation in digestibility or the proportion of cell wall is stage of maturity of the plant (Corbett 1978; Thompson and Poppi 1990). Digestibility only measures the difference between the food consumed and faecal output, and indicates nothing of the processes of digestion (Ulyatt 1970a).

Dry matter digestibility measures many dynamic factors in forage quality and combines in a single numerical value many of the changes which result from forage management, plant growth, and other factors (McCullough 1959; Blaxter 1960). Digestibility is not a basis for the exact prediction of animal performance, but can allow a general appraisal of nutritive value (Blaxter 1960). Environmental factors including heat, drought, and light intensity can affect the digestibility of a forage (Vogel and Sleper 1994). Significant genotype x environment and genotype x management interactions have been demonstrated for digestibility (Hacker 1982).

2.2.4 Maturation of perennial ryegrass

The apparent digestibility of organic matter decreases with advancing maturity (Terry and Tilley 1964; Waite et al. 1964; Cooper 1973; Ulyatt 1981a and b; Hacker 1982; Norton 1982). All parts of the ryegrass plant have a high digestibility at early stages of growth in the spring (Terry and Tilley 1964). A high digestibility is maintained in the spring and this declines as the plant matures over the summer (Table 2.1). This occurs because as the ryegrass plant matures the proportion of stem increases and digestibility of the stem declines. This is associated with the translocation of soluble carbohydrates from stem and leaves to the inflorescence (Norton 1982). The digestibility of the stem falls off at a much faster rate than that of the leaf (Terry and Tilley 1964). The stem is of lower digestibility because it contains higher proportions of the plant structural components cellulose and hemicellulose, which are slowly digested, and lignin, which is indigestible. (Ulyatt 1981a and b; Cooper 1973). The physical resistance to breakdown in the rumen increases. As a result the apparent digestibility, intake, and nutritive value and feeding value all decrease with increasing maturity (Ulyatt 1973; 1981a and b).

The rate of decrease in digestibility is small until seedhead emergence but thereafter declines rapidly (Minson 1960; Waite et al. 1964). Young rapidly growing stem has been found to have a digestibility at least as high as the leaf (Minson 1960; Terry and Tilley 1964). It is only when stems and flowering heads are allowed to become mature that they lead to a marked depression in digestibility (Minson et al. 1960). Armstrong (1964) reported that maximum liveweight gains of animals fed S23 perennial ryegrass hay would be expected if hay was cut just before ear emergence.

Table 2.1 The chemical composition (% DM) and apparent digestibility by sheep of S23 Perennial ryegrass at various stages of maturity (Waite et al. 1964; Armstrong 1964).

Cut	Stage of growth	Crude protein	Readily fermentable carbohydrate	Structural carbohydrate	Lignin	Energy apparent dig. (%)
1	Young leafy	18.5	20.4	37.1	2.7	83
2	Late leafy	15.2	18.8	41.0	3.6	78
3	Head emergence	13.8	18.1	43.3	4.3	77
4	Seed setting	9.6	15.7	52.4	7.3	64

2.2.5 Definition of nutritive value

The nutritive value of forage is defined as the concentration of nutrients in a feed, or animal response per unit of intake (Ulyatt 1981a). Nutritive value is a function of many complex factors, such as the chemical and physical composition of the feed, digestibility, rate of digestion, site of digestion and volatile fatty acid production (Black 1990). These factors also influence intake, so intake and nutritive value are not independent entities (Ulyatt 1970a). The nutritive value depends on the proportion of nutrients digested and on the efficiency with which these digested nutrients are absorbed and utilised within the animal's tissues (Raymond 1969; Corbett 1978; Ulyatt 1981a). Digestibility is a major component of nutritive value (Mott 1959; Armstrong et al. 1964; Ulyatt 1971; 1981a).

Within any sward, forage quality varies not only with genus, species and cultivar, but also with different plant parts, stage of maturity, soil fertility and with local and seasonal conditions (Mott 1959; Norton 1982). Improved herbage varieties even when they are at the same stage of maturity may differ in nutritive value (Minson et al. 1960). Different animals have different nutritional requirements, depending on their species, sex, physiological status, and level of production (Raymond 1969).

2.2.6 Definition of feeding value

Pasture quality can be assessed according to animal production and is a product of intake and nutritive value (Mott 1959; Ulyatt 1970a). Feeding value is defined as the animal production response to the total herbage consumed (Ulyatt 1973; 1981a and b; Laidlaw and Reed 1993). It is a biological assessment of the worth of a herbage in terms of animal production (Ulyatt 1973) and can be assessed in grazing experiments where intake is not limiting (Ulyatt 1981a). The response in animal production will depend on the type of animal, its physiological state, and the environment to which it is subjected (Ulyatt 1970a). The causes of differences in feeding value require measurement, including voluntary intake and the components of nutritive value (Ulyatt 1981a). However, the complexity of the interactions between factors contributing to feeding value (Figure 2.1) can make it extremely difficult to identify specific reasons for differences in performance in a given situation (Black 1990).

Figure 2.1 Factors contributing to feeding value. Adapted from Black (1990).

-
- ♣ Nutrient content – plant species, component parts, stage of maturity and growing conditions.
 - ♣ Pasture components selected by animal – ease of eating, taste, odour, and tactile characteristics of experience.
 - ♣ Amount of each selected component eaten – potential of animal to utilise nutrients, capacity of reticulo-rumen, rate of digestion and outflow of organic matter from rumen and time spent eating and ruminating.
 - ♣ Modification of consumed nutrients by rumen microbes – extent of breakdown of plant protein and proportions of volatile fatty acids produced.
 - ♣ The efficiency of biochemical reactions metabolising absorbed nutrients
-

Animal production can be improved by increasing the nutritive value of pastures. This can be achieved by application of management techniques such as increasing the proportion of cultivars of high nutritive value in the pasture, and through management which promotes pastures which are immature and growing rapidly for maximum nutritive value (Ulyatt 1981a). Implementation by farms depends on economics, such

that the farmer can get a return for his skill and any increased financial input (Ulyatt 1981a).

2.2.7 Breeding for improved nutritive value

The task of the plant breeder

It is the task of the plant breeder to accord priorities to possible objectives and to integrate them into a realistic breeding programme (Easton 1983). The breeder can select for required characteristics within a pool of genetic variation in existing varieties, or widen the genetic base by bringing in new material (Frame 1992). Van Wijk et al. (1993) describes plant breeding as identifying and steering variation in order to produce new varieties that present seed or plant material of superior genetic and physical quality to the farmer. A new cultivar should offer some prospect of advantages over lines in current use (Morley 1978). The commercial breeder is involved in a continuous interaction between variety development, seed production, processing and marketing.

The difficulty which faces both plant breeder and the farmer, is what weighting to give to the components of herbage production and quality. The relative importance of the two characteristics will vary depending on the animal production system in question (Munro et al. 1992). The plant breeder is faced with the complex task of breeding for feeding value to livestock with a variety of needs in addition to breeding for plant production and persistence (Frame 1992). An improvement programme in its early stages is primarily concerned with selection for survival and seasonal production of dry matter (Cooper 1973). A cultivar must have general adaptability to the environment combined with good yielding capacity that confers the ability to persist and yield consistently well (Wilson 1981).

In selection for nutritive value, the first step is to identify the most important nutritional components (Milford 1960; Cooper and Breese 1980). This will depend on the ability of existing varieties to meet nutritional requirements, and on how much genetic variation exists in the characteristics concerned (Cooper 1973). The second step is to

develop rapid and reliable screening tests that can deal with the large number of small samples involved in a breeding program (Cooper and Breese 1980). Finally, the resulting selection lines or potential varieties must be critically assessed in terms of animal performance under grazing. The process of plant breeding can take up to 15 years or more from the initial cross to the fully tested variety reaching the farm in a seed mixture (Frame 1992). The ultimate test of a new variety is performance under commercial farming conditions (Frame 1992).

Past progress

Reed (1994) reviewed improved grass cultivars for increasing milk and meat production and reported that improvement programmes have raised the feeding value of pasture species considerably. Vogel and Sleper (1994) have reported that cultivars selected for improved quality have usually resulted in a higher profitability than similar improvements in forage yield. In contrast, Smith et al. (1997) believes that there have been few successful programmes selecting forage plants with improved nutritive value, despite the implications of improved forage quality for production. This lack of progress was attributed to differences in opinion on the relative importance of improving individual traits relating to nutritive value. Van Wijk (1993) and Hutchinson and Clements (1987) believe that purposeful breeding for improved nutritive value has not resulted in an appreciable range of commercially important varieties.

Nutritive value was not included in any of the breeding objectives by Australian plant breeders (Hutchinson and Clements 1987) and only rated 13th on the selection criteria in one survey (Cunningham et al. 1993b). However, when a Delphi survey aimed specifically at nutritive value traits was conducted, increasing digestibility was ranked the most important goal for improving milk production (Smith et al. 1997) and for improving liveweight gain and wool production (Wheeler and Corbett 1989). Increasing non-structural carbohydrate, improved rate of digestion, ease of comminution and high protein content were also important. Similar rankings were achieved from Australian, New Zealand, UK and American scientists and plant breeders (Smith et al. 1997).

Selection pathways

There is considerable variation within species for characteristics associated with quality and this variation may be exploited by the plant breeder to overcome existing limits to animal production (Hacker 1982). Which component of quality is the most useful criterion of nutritive value is difficult to decide because they are highly correlated with each other (Armstrong 1964). Some nutritive value characters can be measured much more simply and rapidly than others, allowing a greater intensity of selection for the easily measured traits (Clements 1970). Generally, the greater the number of selected characters, the smaller is the improvement in each per unit time or alternatively the larger is the population required for screening to give the same improvement per unit time (Hutchinson and Clements 1987). Programmes for improving the nutritive value of herbage are shown in Figure 2.2.

Figure 2.2 Alternative programmes for improving the nutritive value of herbage. Adapted from Hutchinson and Clements (1987).

-
- ♣ Direct selection for nutritive value, for example digestibility, using traditional breeding methods
 - ♣ Breeding for agronomic characteristics which may improve nutritive value under grazing
 - ♣ Breeding to reduce the effects of biological and chemical agents that may impair the performance of an otherwise nutritious species.
 - ♣ Managing existing forages better
 - ♣ Molecular genetics
-

The plant breeder needs to produce cultivars not only with high digestibility, but also with high intake characteristics and high feed utilisation for grazing (Cooper and Breese 1980). A selection index can be produced to select within a species to improve the quality of a variety (Ulyatt 1970a). It is essential that the selection index is simple to measure (Ulyatt 1970a). General between-species indices between species are not appropriate. Species have a wide range in morphology, anatomy and chemical composition, therefore it is likely that differences in quality are caused by different factors (Ulyatt 1970a). Indices that have been suggested include high digestible dry

matter content, high content of soluble carbohydrate and crude protein (Armstrong et al. 1964; Castle and Watson 1971). Marvin et al. (1997) demonstrated significant variation in perennial ryegrass for organic matter degradation, neutral detergent fibre and for the production of volatile fatty acids upon incubation in the rumen fluid.

Differences between species

Large differences in nutritive value have been demonstrated between species. Fraser et al. (1997b) has reported significant differences in lamb liveweight gains, carcass weights and wool growth rates in lambs grazing white clover, lotus, chicory and plantain. Perennial ryegrass has a relatively low fermentation rate and because of its anatomical structure, is very resistant to mechanical breakdown by chewing. Thus feed remains in the rumen for a long time, resulting in lower intakes than in the case of white clover (Ulyatt 1981b). Superior liveweight gains of sheep grazing clover and short rotation ryegrass (perennial x Italian hybrid) over perennial ryegrass have been well-documented (Rae et al. 1961, 1964; Ulyatt 1970b, 1971). The superior quality of white clover and short rotation ryegrass is primarily due to their low structural carbohydrate content enabling faster breakdown in the rumen, leading to a higher intake of readily fermentable carbohydrate and protein. Even at similar intakes, clover is utilised more efficiently than ryegrass for energy and weight gains (Rattray and Joyce 1974).

2.2.8 Selecting specifically for improved digestibility

Potential

There is wide agreement in the literature that selection for high digestibility is a valid breeding objective in perennial ryegrass (Cooper et al. 1962; Dennis and Fransen 1986). It is believed that small changes in organic matter digestibility can have a significant impact on animal production (Blaxter 1960; Wheeler and Corbett 1989; Vogel and Sleper 1994) and the profitability of production systems (Vogel and Sleper 1994). To date only a limited number of forage cultivars with improved digestibility as validated in animal trials have been released (Vogel and Sleper 1994). This is thought to be due

to breeders not recognising the economic value of selecting for improved digestibility or believing other traits were more important. There has also been a lack of commitment to animal evaluation trials to validate the results of laboratory and small plot research (Vogel and Sleper 1994).

Selections of bermudagrass (*Cynodon dactylon* (L.) Pers.), switchgrass, (*Panicum virgatum* L.), wheatgrass (*Agropyron*) and lucerne (*Medicago sativa*) have demonstrated improved digestibility and increased animal production (Vogel and Sleper 1994). Since management costs will be identical among similarly yielding cultivars of a species except for the possible differences in seed cost, genetic improvement in digestibility that leads to greater liveweight gains can be considered to be 100% profit (Vogel and Sleper 1994). Hodgson (1981) proposed yield x digestibility profiles would be useful in the future to relate the performances of different plant genotypes to the nutrient requirements of different classes of animals.

The relationship between digestibility and herbage intake

Intake accounts for at least 50% of the variation observed in feeding value (Ulyatt 1973 1981b). In general digestibility and intake are the most serious quality limitations to animal production from pasture (Hacker 1982). Rate of intake depends on digestibility, chemical composition, palatability, grazing pressure and the animal's response to the environment (Mott 1959). Troelsen and Cambell (1969) reported that herbage intake declined by $1.5 \text{ g/kg}^{0.75}$ of body weight per unit decrease in digestibility percentage for grass hay (average digestibility was 58.9%).

Since greater digestibility increases intake, as well as increasing the amount of nutrients extracted per unit of forage eaten, differences in herbage quality between cultivars offer greater potential for increasing animal output than do differences in herbage yield (Snaydon 1979). The primary factor governing the nutritive value of herbage is its digestibility, which not only determines the proportion of the feed which can be utilised by the animal, but also greatly influences the amount eaten (Cooper et al. 1962).

When a wide range of forages are compared there is an overall positive correlation between digestibility and intake; the more digestible the forage, the more is eaten (Hacker 1982). Indoor feeding trials have shown that the relationship between digestibility and voluntary intake may be linear up to 80% digestibility (Munro and Walters 1986). Hodgson et al. (1977) found that digestibility exerted a dominant influence on herbage intake by beef cattle. Other reports suggest that above 70 to 75% digestibility intake may increase but the animal response will diminish or cease since the level of energy intake exceeds the level of energy required (McCullough 1959; Ulyatt 1970a).

There can be marked variation in intakes and animal performances at the same levels of digestibility. Different forage species may show different intake levels at the same digestibility (Hacker 1982). Ryegrass pastures of similar digestibility can have quite different feeding values depending on the season of the year (Reed 1978). Lower liveweight gains in autumn have been reported to have been associated with a reduction in voluntary intake (Reed 1978). Laredo and Minson (1973) reported that the intake of leaf was 46% higher than that of stem, despite similar digestibility in tropical grass species. The higher intake of the leaf fraction was associated with a shorter retention time in the reticulo-rumen which appeared to be caused by the large surface area of the leaf fraction available for bacterial degradation. Therefore, there is potential for breeding for intake in forage crops independently of digestibility (Walters 1971). There needs to be caution when using digestibility, growth stage or leafiness as indicators of the nutritive value of grass crops (Walters 1971).

Expected gains in animal performance

McCullough (1959) suggested that when animal and plant variability is considered, differences of 5% in digestibility would, on the average, produce highly significant changes in animal response. Clark and Wilson (1993) used a dairy farm simulation model (UDDER) to predict that an increase of 5% digestibility of pasture would increase milkfat per cow and per hectare by 3.9% . The gross margin was predicted to increase by 5.7% if the improved cultivar lasted for 3-5 years to cover the costs of

establishment. The digestibility of intensively managed pasture normally lies within the range 60% – 80% (Freer 1981). At the lower level, the intake of digestible energy would support little more than maintenance (Wheeler and Corbett 1989; Freer 1981). Wheeler and Corbett (1989) predicted that an increase in digestibility from 65% - 70% could double the rate of liveweight gain provided that there is sufficient forage available to allow the increase in intake associated with the higher digestibility. Blaxter (1960) estimated the liveweight gain expected from feeding hays varying in digestibility of energy (Table 2.2). Freer (1981) estimated that for a 50 kg sheep its herbage intake would increase by about 20-25 g DM for each increase of one unit of digestibility. The digestibility of herbage intensively managed pastures normally lies within the range 65-80% during the growing season. Freer (1981) suggests that at the upper level lambs might be expected to gain 300 g/day.

Table 2.2 The effect of hay digestibility on estimated liveweight gain of sheep fed ad lib. Adapted from Blaxter (1960).

Feed	Apparent digestibility of energy	Observed gain/day g/kg W ^{0.73}	Estimated gain for a 30 kg sheep (g/day)
Poor hay	44.4	+ 0.7	8.4
Medium hay	59.6	+ 5.4	64.7
Good dried grass	74.3	+ 9.4	112.6

Natural variation

In selecting for digestibility plant breeders are interested in the availability of genetic variation and efficiency of individual plant selection (Dennis and Frandsen 1986). There is sufficient additive genetic variation available for digestibility in perennial ryegrass to warrant improvement by suitable selection methods (Dennis and Frandsen 1986). In general digestibility of forage grasses is believed to be under the control of a large number of genes, but there are instances where single major genes control a major

component of digestibility (Hacker 1982; Vogel and Sleper 1994). Differences in digestibility are inherited in a quantitative manner (Vogel and Sleper 1994). Differences in herbage digestibility can arise from different rates of development and different digestibilities of the component fractions, and these are to a considerable extent under genetic control (Minson et al. 1964; Raymond 1969).

Differences in digestibility within a species can in some instances be defined in terms of differences in leaf to stem ratio and in chemical composition (Ulyatt 1981b). Much of the variation observed within a population of plants is due to differences in maturity (Walters et al. 1967; Raymond 1969; Van Wijk 1993). Small differences in the digestibility of a given forage cultivar cut on the same date in different years may be due to the delayed onset of active spring growth in a late season, or to differences in leaf percentage (Raymond 1969). Significant differences in digestibility between progenies over maturity may have been due in part to differences in earliness of flowering (Dennis and Frandsen 1986). This variation may finally turn out to be negligible when truncated at levels of maturity or heading date (Van Wijk 1993). Van Wijk (1993) believes that once the gross variation due to maturity classes has been taken care of by maturity grouping there is little net variation remaining to be measured accurately within these maturity groups. Variation in digestibility between cultivars still remaining after reproductive growth (regrowth) may be a result of inherent differences (Walters et al. 1967).

Heritability estimates

Heritability is the proportion of variation that is genetic. Cooper et al. (1962) reported a range of digestibility values of 63 – 84% within a single ryegrass cultivar (S.23). The heritability estimated from parent-progeny correlations was not significant because all parents used for the crossing were of high digestibility. Dennis and Frandsen (1986) have estimated the narrow-sense heritability for the genetic variation in digestibility of perennial ryegrass as 0.34 - 0.57. They also reported that the rate of decline in digestibility was under genetic control with a heritability value of 0.64 for perennial ryegrass (Dennis and Frandsen 1986). Hacker (1982) quoted a heritability of 0.42% for

perennial ryegrass. At this level of heritability some advance under selection can be expected in species already of high digestibility such as perennial ryegrass, particularly when at a fairly mature stage at or after flowering. The margin for improvement appears greater, however, in species at present of lower digestibility such as cocksfoot (Cooper et al. 1962).

Selection pathways

As pasture matures, digestibility declines due to the decreasing proportion of the younger more digestible leaf and an increase in lignification of the stem with flowering and maturation. Therefore improvement in digestibility by breeding could potentially operate through a number of indirect pathways including later flowering, greater leafiness, reduced lignification or high proportion of cell contents (Hacker 1982; Dennis and Frandsen 1986; Vogel and Sleper 1994). Selection for a slow rate of decrease in digestibility of primary growth could be exploited as a means of improving digestibility (Dennis and Frandsen 1986). The extent to which digestibility of a forage is related to leafiness will depend on the relative digestibility of leaf and stem (Hacker 1982).

These factors can all be considered to be components of whole plant digestibility and successful selection for any single factor will improve whole plant digestibility. Organic matter digestibility is the only criterion that integrates all components of digestibility. If there is genetic variation for any of the components of digestibility, the digestibility of the forage is likely to be improved by breeding for higher organic matter digestibility (Vogel and Sleper 1994). Therefore, by attempting to improve digestibility by selecting for only one component such as neutral detergent fibre, the potential gain is limited by the genetic variation for that trait alone (Vogel and Sleper 1994). Selection for digestibility can be based on material either at a particular calendar age, or physiological age related to flowering (Hacker 1982).

Measurement of digestibility

Breeding for improved digestibility first became feasible when reliable, repeatable, *in vitro* dry matter digestibility methods were developed (Vogel and Sleper 1994). Plant breeders are now able to screen large numbers of plants using this technique. Reliable *in vitro* techniques have revealed useful genetic variation between varieties and between individual genotypes for overall digestibility (Cooper 1973). *In vitro* organic matter digestibility can be used as a practical measure of nutritive value for plant improvement and as a primary screening characteristic (Ayres 1991). However, there is a need for formal standardisation of the *in vitro* digestibility procedure to safeguard the integrity of digestibility data (Ayres 1991). Spectroscopy (NIRS) is a rapid technique for prediction of nutritive value and has been used for prediction of digestibility (Cunningham et al. 1994). It could be used to reduce large plant populations to a manageable size for more complex chemical analyses (Hutchinson and Clements 1987).

Correlations with other plant characteristics

Desirable characters can be negatively correlated, so that selection for one of them leads to unfavorable correlated responses in another (Clements 1970). Selection for higher whole plant digestibility is sometimes negatively correlated with plant yield, but there is thought to be sufficient variation in both characters that improvement will not lead to a decrease in yield or environmental resistance (Hacker 1982; Dennis and Frandsen 1986; Clark and Wilson 1993). Where negative correlations exist, acceptable lower limits for the related characters need to be defined and some compromise may be necessary when selecting for the major character (Clements 1970). There is usually a strong negative correlation between digestibility and lignin, cell wall content and neutral detergent fibre content (Hacker 1982).

2.2.9 Perennial ryegrass cultivars selected for improved nutritive value

Improvement in yield and persistence

Improvement in annual dry matter yield is estimated as approximately 0.1% per year by grass breeders in the United Kingdom (Clark 1993), or 0.6% per year from new ryegrass varieties coming on to the NIAB Recommended List (Frame 1992). In absolute terms only small differences exist between modern and old cultivars however they are assessed (Clark 1993), and it appears that the grass breeder has had limited success in increasing above-ground herbage mass (Clark 1993). Herbage production is not always a reliable predictor of animal performance because it does not account for differences in nutritive value. Thom et al. (1998) reported that total annual yields, season yields and persistence of newly released cultivars was no better than that of a standard cultivar (Yatsyn 1) over three years.

Flowering date

Earliness of flowering is generally negatively correlated with digestibility (Dennis and Frandsen 1986). Late flowering ryegrasses produce vegetative growth in late spring when other cultivars have flowered (Easton 1983; Fraser 1997a). Therefore when compared at the same calendar date, late flowering varieties have the higher digestibility and leaf to stem ratio because of their slower rate of development (Minson 1964; Ramond 1969; Van Wijk 1993; Vogel and Sleper 1994). However, when compared at the same stage of morphological development, early varieties are usually more digestible (Hacker 1982). When forage of cultivars differing in earliness of flowering are offered simultaneously during stem elongation to flowering, sheep generally eat more of the late types (Simon and Daniel 1981). Therefore the stage of maturity of pasture and the liberty of the animals to choose are factors that strongly affect the results of experiments comparing cultivars (Simon and Daniel 1981).

Evidence presented by Munro et al. (1992) showed that lamb production from early (Aurora and Frances) and intermediate-flowering (Talbot) cultivars was as good as that

from late-flowering perennial ryegrass (Melle) grazed as grass-only swards in the United Kingdom. Over two years, total annual lamb production per hectare from grass-only swards of Aurora was 19% more than that from Frances because of better liveweight gain/lamb, despite similar herbage productivity.

Tetraploid verses diploid

Perennial ryegrass is naturally diploid, containing 14 chromosomes. Tetraploidy is the artificial doubling of the chromosome number using colchicine and was first achieved in the *Lolium* species in the 1930's (Easton 1983). Tetraploid plants are bigger and fleshier, but the water content is higher so that dry matter yields show little if any improvement (Easton 1983). Even at the same stage of maturity tetraploids have a higher digestibility than diploids (Raymond 1969; Davies et al. 1991).

Castle and Watson (1971) compared S24 (diploid) and Reveille (tetraploid) perennial ryegrass. The average output of milk per hectare was approximately 4% higher from the Reveille than from the S24 swards. Hageman (1993) demonstrated higher herbage intakes of 0.6 kg/cow/day and significantly higher average daily production of fat and protein from cows grazing tetraploid cultivars (Madera and Condesa) compared to a diploid cultivar (Wendy). Swift et al. (1993) and Vipond et al. (1993) reported tetraploid swards (Condesa) produced 16% greater overall lamb output than the diploid swards (Contender) due mainly to a 10% higher carrying capacity.

Shear strength

Evans (1964) indicated that leaf strength would be a useful screening technique for high nutritive value. Mackinnon et al. (1988) reported that leaf shear strength was a heritable trait and could be measured using a Warner-Bratzler machine (Easton 1989). Inoue et al. (1989; 1993a and b) selected perennial ryegrass for high or low leaf shear strength. The line selected for reduced shear strength was consumed in greater quantities and was digested more rapidly in the rumen than perennial ryegrass selected for high leaf shear strength. The lower shear strength was a result of decreased sclerenchyma content that

would result in easier breakdown during rumen digestion (John et al. 1989). However, under field conditions there was no significant differences in intake, rumen retention times or liveweight gain of sheep fed the two lines (Inoue 1993b). Therefore it was concluded that leaf shear breaking load was not an appropriate criteria on which to select for improved feeding value.

Water-soluble carbohydrate content

An aim at the Welsh Plant Breeding Station has been to improve herbage quality by increasing water-soluble carbohydrate (sugars) content. This can result in better acceptability and digestibility of the herbage, with increased animal intake and consequently more efficient animal production (Humphreys 1989; Frame 1992). Aurora and Cariad cultivars of perennial ryegrass exhibited higher water-soluble carbohydrate concentrations than other cultivars particularly over summer. This resulted in an improvement in dry matter digestibility of between 2 and 6% (Radojevic et al. 1994). This could improve digestibility during summer when feed is declining in quality.

2.2.10 Perennial ryegrass cultivar comparisons

United Kingdom and Europe

Davies et al. (1989a and b, 1991, 1992, 1993) demonstrated significant differences in sheep production from early flowering (Aurora), late flowering tetraploid (Meltra) and late flowering (S.23) perennial ryegrass cultivars under continuous stocking management. Lamb production was positively correlated with organic matter digestibility. Mean total annual lamb production per hectare from Aurora and Meltra was 16% and 13% more than that from S23 from 1985 to 1987 (Davies et al. 1989a). From 1988 to 1990 lamb production was 7% and 16% more than that of S.23. Over three years organic matter digestibility of the later flowering tetraploid Meltra was higher than that of the diploids S23 and Aurora (Davies et al. 1993). Evans et al. (1979) reported 14% higher liveweight gains from the cultivar Mascot than from S.23 perennial ryegrass. The better liveweight gains reflected greater intake and greater efficiency of

feed use by the animal. This was related to higher leaf and lower dead material contents in Mascot with correspondingly higher digestibility. Hazard (1998) reported significant differences in herbage intake between four cultivars of perennial ryegrass but found the order was management dependent. The ingestibility was positively correlated with the lamina to pseudostem ratio, while digestibility was negatively correlated with the proportion of dead leaves in the fresh forage.

New Zealand

Quality was reported to be higher in Ariki ryegrass (perennial x short rotation hybrid) than in perennial ryegrass (Barclay 1963). Lancashire and Ulyatt (1975) showed consistent but non-significant increase in liveweight gain in three trials comparing a low cellulose selection of Ariki hybrid perennial ryegrass with the higher cellulose Ariki ryegrass. The results suggested that the feeding value of Ariki ryegrass was only marginally improved through selection for low cellulose level. Significant differences in sheep liveweight gains (Harris and Johnston 1967; Ulyatt 1973; Ulyatt et al. 1974) and in milk yields of cows (Wilson 1966; Wilson and McDowall 1966; Wilson and Dolby 1967) from perennial and hybrid ryegrass cultivars have been reported.

However, the endophyte status of these pastures was unknown in the above comparisons. The presence of endophyte in perennial ryegrass cultivars was not linked to persistence or to animal health disorder such as ryegrass staggers until 1981. Results from these comparisons were probably confounded by reduced pasture production caused by Argentine stem weevil damage and the occurrence of ryegrass staggers on some cultivars, and therefore results must be accepted with caution. All comparisons of perennial ryegrass cultivars in New Zealand must be interpreted with respect to their endophyte levels. The perception of genetic differences between cultivars in field performance has been impaired by the presence of the *Lolium* endophytes (Easton 1983). McCallum and Thomson (1994) found no effect of ryegrass cultivar on calf liveweight gains or milk production or composition between Yatsyn-1, Embassy, Vedette or Pacific ryegrass cultivars of known endophyte status. Lamb carcass weight and final hogget liveweight were superior from Marsden and Greenstone hybrid which

contained a higher proportion of leaf to stem compared with Pacific perennial ryegrass pastures (Ryan and Widdup 1997).

Perennial ryegrass compatibility with clover

Differences between cultivars in lamb output and herbage characteristics are often more pronounced in grass/clover swards (Munro et al. 1992; Davies et al. 1993). In mixed pasture comparisons, the variability between grass cultivars in either seasonal growth rhythm and/or morphology, has influenced the growth of companion white clover (Reed 1994). Davies et al. (1993) reported that the tetraploid variety Meltra was more compatible with clover, which resulted in higher sheep liveweight gains. Elgersma and Schlepers (1997) reported that a prostrate diploid perennial ryegrass variety formed a dense sward with less clover and less weeds than a tetraploid or erect diploid ryegrass variety. However, these differences were not reflected in differences in animal performance or net energy production.

2.2.11 Adoption of new cultivars by farmers

New Zealand farmers tend to have a better knowledge and higher use of older cultivars of perennial ryegrass (Belgrave et al. 1990). In the late 1970's Ruanui comprised over 70% of retail sales of perennial ryegrass because of its reputation to be tolerant of poor management and low soil fertility (Lancashire et al. 1987). Initiative in plant improvement may not be supported if cultivars released earlier have not been well accepted (Reed 1987). The rate of pasture reseeding by New Zealand farmers is low. One survey covering 1977-81 for central New Zealand suggested that farmers were renovating or renewing only 4-6% of their existing pasture annually (Lancashire 1985). Many farmers believe that improved management of existing pasture will generate greater returns than introducing new cultivars (French and Simmonds 1985). Snaydon (1979) estimated that if pastures are resown at five-year intervals then output must increase by 30% to cover additional costs.

Some farmers also believe that the advantage of sowing a new cultivar would not exceed the disadvantage of cost, extra worry and physical effort (Snaydon 1979; French and Simmonds 1985). Ease of management is important because it determines the extent to which the farmer can achieve the potential of the cultivar, and also determines the requirements for labour, technical skills and additional inputs (Snaydon 1979). Farmers require more animal performance data and economic analysis of costs versus benefits for new cultivars (French and Simmonds 1985). There have been very few long term, large-scale system comparisons of animal production from contrasting cultivars in New Zealand (Lancashire 1985). To improve the rate of herbage cultivar adoption there must be better communication between farmers, private seed companies and agricultural scientists (French and Simmonds 1985; Cunningham 1994). This would strengthen breeding programmes by combining scientific expertise and support research with breeding, seed production, and commercialisation skills and ensure a balanced focus on industry and market needs (Cunningham 1994).

2.3 THE ANIMAL EVALUATION PROCEDURE FOR NEW PERENNIAL RYEGRASS CULTIVARS

2.3.1 Importance

It is strongly recommended in the literature that new cultivars be evaluated by the grazing animal and under conditions in the field in which they must perform (Morley 1978; Hodgson 1981; Ulyatt 1981a; Ceccarelli et al. 1992). 'This is to ensure that evidence comes from grazed swards and the grazing animal accepting that this may increase the complexity and reduce the precision of the measurements' (Hodgson 1981). The response of the animal to the pasture is of over-riding importance (Rae et al. 1961). Attempting to breed forages for improved forage quality without conducting animal evaluation trials can lead to erroneous conclusions (Vogel and Sleper 1994). Animal evaluation must occur early on in the breeding process (Mochrie et al. 1981; Hodgson 1981). The Welsh Plant Breeding Station developed a five phase evaluation process where animals are involved from phase 2, with animal production being assessed in phases 4 and 5 (Wilkins 1986). A comprehensive animal evaluation process (Figure

2.3) will take less than 10 years, and will depend on the labour and resources available (Mochrie 1981).

An independent coordinated testing network must be available for testing new cultivars (Reed 1987). Small-plot studies should be supported with animal production trials to maintain credibility and remain focussed on feasible, commercially relevant goals for plant improvement (Reed 1994). Issues such as compatibility with companion clover species, toxin effects and compensatory gains should be examined (Reed 1994). Animal production trials are the most direct means of detecting antiquality factors such as endophyte in promising cultivars, and assess nutritive value continuously, providing a basis for economic assessment and testing persistence under commercial conditions (Laidlaw and Reed 1993). Animal performance results also have a role in technology transfer and can result in a more rapid adoption of a new improved cultivar (Laidlaw and Reed 1993). Differences in comparative feeding value can be assessed by grazing trials where a standard herbage is included and where intake is not limited by herbage availability (Ulyatt 1981b). Average daily gains are the best estimate of forage quality and will show if selection for improved digestibility has resulted in improved animal performance (Vogel and Sleper 1994).

Figure 2.3 A practical four-stage model for the animal evaluation of a new cultivar. Adapted from Mochrie et al. (1981).

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- ♣ Phase I - in vitro organic matter digestibility for quality.
 - ♣ Phase II - animal preference to assist cultivar selection and measure quality.
 - ♣ Phase III - qualitative animal response including daily gains and quality of consumed forage.
 - ♣ Phase IV - quantitative animal response including production/ha from optimal management schemes fertility, grazing method, and some evaluation of persistence.
-

2.3.2 Cutting Trials and Indoor Feeding Trials

It is widely agreed in the literature that comparative measurements of herbage production in cutting trials are unlikely to be a reliable guide to performance under grazing (Morley 1978; Hodgson 1981; Wilkins 1986). Plant genotypes can vary in growth habit under cutting and grazing management (Hodgson 1981). The grazing animal places unique pressures on pasture plants (Figure 2.4). Grazing is uneven leaving swards more variable than those which have been defoliated by mowing machines (Morley 1978). Changes in management practices can drastically alter the relative performance of cultivars (Casler et al. 1998). Likewise, indoor feeding trials lack realism because of the removal of many of the essential features of grazing, especially animal selection, treading and the return of nutrients in excreta (Evans 1979). It is unwise to predict animal performance under grazing conditions from indoor measurements (Hodgson 1981).

Figure 2.4 Effects of the grazing animal on pasture plants. Adapted from Casler et al. (1998).

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- ♣ Defoliation of plant tissue, which reduces photosynthetic capacity and may reduce root development and carbohydrate storage
 - ♣ Selection of plant parts and plant species
 - ♣ Trampling, which damages plant tissue, increases soil bulk density, and slows water infiltration
 - ♣ Excretion, which affects plant palatability and nutrient cycling
-

2.3.3 Precision of Animal Evaluations

Feeding value trials have been criticised for their relative lack of precision, applicability restricted to conditions similar to those of the trial, and requirement of special resources (Laidlaw and Reed; 1993). Feeding trials have lower precision than cutting trials due to the large variation between animals and grazing effects on large swards increasing the coefficient of variation (Laidlaw and Reed; 1993). Animal performance trials are only

indicators of potential value of the cultivar in actual farming practice because measurements are made under controlled conditions and may not reflect commercial farming grazing management (Hodgson 1981). Conflict can arise between the objective of maximising the precision of an experiment and the value of the results of such an experiment (Morley 1978). It may be much more valuable to establish a difference of $P < 0.10$ over several sites than to have a statistically more precise experiment the results of which are of little value for generalisation. The most sensitive comparisons should be associated with decisions where errors could have the most serious consequences (Morley 1978). The ultimate test of cultivars should be their performance in a production system, but if adequate control is not exerted over the system then the interaction between management and herbage growth could mask or enhance actual differences which exist between cultivars (Laidlaw and Reed 1993).

2.3.4 Resources

Evaluation through animal production must involve compromises between the desirability of studying a total system on an adequate scale and the resources available (Morley 1978). For financial and logistical reasons, few grazing experiments have been conducted where cultivar differences in herbage yield and quality have been evaluated in terms of milk, meat or wool production (Evans 1979). Measurement of feeding value is claimed to be expensive compared to herbage yield cutting trials, but when considered in the context of the cost of breeding a new cultivar they are not excessively costly (Laidlaw and Reed; 1993). Problems of time, resources, and personnel may become so great as the complexity increases that the number of treatments that can be investigated must become seriously restricted (Morley 1978). A small experiment may be more useful than a large and complex one, because in large experiments it may not be possible to measure all components of the grazing system such as small but perhaps important changes in botanical composition accurately enough to enable interpretation to be unequivocal (Morley 1978).

2.3.5 Design

The objectives of a particular experiment will determine which measurements of the grazing system are appropriate and necessary. As the experiment progresses, new and previously unsuspected opportunities or limitations may come to light (Morley 1978) and reappraisal is necessary to avoid continuing collecting data of doubtful relevance (Morley 1978). With a given liveweight gain response, more detailed measurements are required to understand why the observed level of animal production was obtained and why it may vary within and between years, and the relevance of the information to other situations and areas (Corbett 1978). Measurements of animal production are not usually sufficient by themselves to achieve an understanding of the complexities of a grazing system. Many attributes of pasture and of animals, as well as of animal products should be measured (Morley 1978).

Measurements of animal performance require large field plots and require adequate replication of treatments for accuracy. 'Measurements of herbage intake could be made on areas of about 0.1 ha for sheep, or 0.5 ha for cattle and measurements of animal performance would require areas about five times larger' (Hodgson. 1981). It is desirable that treatments include a control/standard cultivar (Ulyatt 1981a; Reed 1994). Controls should be the most popular and the most promising alternative cultivars for the particular situation irrespective of which company may have bred them (Reed 1994). Comparative assessments of cultivars need to continue over a number of years to enable sampling of a range of yearly weather (Morley 1978; Davies et al. 1989a).

2.3.6 Measuring animal performance and herbage intake

The grazing experiment can measure the herbage consumed (quality) and measure the number of animals which the pasture will carry (quantity) (Mott 1959). Even when animals are uniform with respect to age, liveweight, previous treatment and physiological state, there will be considerable variability in performance (Corbett 1978). There is also great variability between animals in herbage intake (Ulyatt 1970a). The coefficient of variation in herbage intake and production is unlikely to be less than 10%,

and values of about 20% can be expected (Corbett 1978). Animals may be allocated to treatments by complete randomisation or stratified randomisation (Robards 1981). Stratified randomisation will take into account factors such as sex, liveweight and previous history. The number of sheep in each plot should be determined by the known or expected variation in the parameters to be measured and the size of differences that can be accepted as a significant treatment effect (Robards 1981). A minimum group size of four animals per plot will ensure that changes in animal behaviour will not prejudice the way in which the plots are grazed (Robards 1981). Animals that escape should be excluded because of their behaviour (Corbett 1978).

Errors in liveweight gain can arise from fluctuations in gut fill. The liveweight of cattle can vary by several kilograms due to day to day and within day changes in gut fill, to variation in the quantity and quality of feed eaten and the amounts of water drunk (Corbett 1978). The quantity of feed eaten, reflecting the amount available on the pasture and the time it was eaten in relation to the time of weighing, have major effects on fill and observed liveweight. Large differences are observed in liveweight from animals grazing at the beginning and at the end of a rotational grazing system (Corbett 1978). Measurements of liveweight gain should be made with groups of animals that are as large as possible (Mott 1959; Corbett 1978). Physical interference and disturbance of normal behaviour patterns caused by experimental procedures will affect animal performance (Corbett 1978). Animals on all treatments must be weighed at the same time, and disturbance of normal behaviour must be avoided (Mott 1959).

The measurement of herbage intake by grazing animals is difficult. The use of oesophageal fistulate animals and indigestible markers such as chromic oxide are imprecise and are labour-intensive (Wilkins 1986). The combined coefficient of variation in estimating digestibility by oesophageal fistulates and by estimating faecal output is about 16%. In addition there will be real differences in intake between animals of similar type (Corbett 1978). Fistulated and non-fistulated animals should not differ in grazing behaviour or diet composition if they have a similar history and nutritional background. Established routines should be used for handling fistulated animals and in the collection of extrusa samples (Forbes and Beattie 1987). Intake

alone cannot account for all the variation observed in liveweight gain (Ulyatt 1970a). Neither intake nor digestibility can be used separately to make comparisons between cultivars (Heaney 1970).

2.3.7 Sources of error

The main source of error in the measurement of animal production is the variability between animals, especially over short periods of time (Mott 1959). Experimental errors are increased when there are variations in grazing pressure, failure to take into account differences in previous treatment of animals and weighing errors (Mott 1959). 'Animal production tests are unlikely to be sufficiently precise, nor will the differences be large enough, to justify more than an occasional asterisk opposite the mean square for cultivar. Differences as high as 10 percent are unlikely to be statistically significant, yet they could be of great economic importance' (Morley 1978).

Error can arise from sampling variation due to heterogeneity of site and differential grazing within plots due to variations in botanical composition, fertility and water relations (Morley 1978). Measurement error can arise from observer or equipment bias, and lack of precision and consistency from observers or equipment (Morley 1978). Observers should understand the importance of their observations so they are motivated to maintain mental accuracy. The precision of individual measurements can be quite low but increasing the number of measurements can compensate for this. Logistical problems arise from the relationship between the area to be covered, equipment, observers and the time available (Morley 1978). Sampling should be carried out over short intervals to avoid drifts in time and to accommodate measurements within the total programme. Replication in the experiment relies on establishing a biological and statistically acceptable balance between the cost of increasing the number of plots, the number of animals, the need to adequately account for variation in pasture composition, soil type and topography of the site (Robards 1981).

2.3.9 Monocultures verses mixed swards

A grass cultivar may be evaluated as mixed sward with legumes as a moderately complex community, or a pure sward with applications of nitrogenous fertiliser (Minson et al. 1960; Morley 1978). In practice there is often weed invasion which requires analysis of botanical composition to distinguish components (Laidlaw and Reed 1993). Weed ryegrasses germinating from the seed bank may not be distinguished from the sown cultivar and may require polyacrylamide gel electrophoresis for identification (Laidlaw and Reed 1993). Pure swards are sown to avoid complications due to varying percentages of clover in different treatments (Minson et al. 1960). An argument for evaluation of mixed swards is that animals on farms will have to select and harvest their diet from a mixed population of plant species and plant components (Corbett 1978; Freer 1981). Most cultivars are tested as monocultures early in the programme that may be extended later to include mixtures to investigate compatibility with legumes.

2.3.10 Grazing management

Cultivar comparisons should be carried out by allocating equal herbage allowances in rotationally/intermittently grazed swards or by maintaining constant sward height in continuously stocked swards (Laidlaw and Reed 1993). Put and take management is appropriate in maintaining a constant sward height (Wilkins 1986) accepting that farmers do not do this on the farm (Morley 1978). In continuous stocking management levels of intake and animal performance are related to sward height or herbage mass (Wilkins 1986). The grazing animal can be a factor affecting nutritional value of pastures. Selective grazing results in a gradual increase in the proportion of the least digestible plant fractions (Milford 1960; Raymond 1969). Using rotational or continuous stocking management has a minor influence of the performance of grazing animals (Pavlu and Velich 1997). In continuously stocked experiments, treatment effects on the animals must be assessed from plot means and not by analysis of individual results (Corbett 1978). In contrast, it is not biologically or statistically sound to classify plots as replicates under rotational grazing management, particularly when the cycle of rotation may take several weeks to complete (Robards 1981). Leader-

follower grazing can be used when high production per animal is required for top grazers and production per animal is satisfactory for bottom grazers (Blaser et al. 1959; Minson 1990). A higher proportion of stem will be left in the follower swards resulting in lower digestibility and depressed intake and subsequent liveweight gain when swards are again grazed (Mott 1959; Minson 1990).

2.4 THE PERENNIAL RYEGRASS/ENDOPHYTE ASSOCIATION AND ITS EFFECT ON ANIMAL PERFORMANCE

The *Neotyphodium* endophyte found in perennial ryegrass is in a mutualistic relationship with its host, in which both the fungus and grass benefit (Latch 1994). The fungus benefits by being protected within the plant and by dissemination through the seed (Latch 1994). The plant benefits by having increased tolerance to stress such as drought and increased resistance to insect attack, improving the overall persistence of the grass (Siegel et al. 1985; Powell et al. 1993b; Latch 1994; Familton et al. 1995). *Neotyphodium lolii* protects against attack by Argentine stem weevil (*Listronotus bonariensis*) which is one of New Zealand's major pasture pests (Fletcher et al. 1990). Endophyte-infected ryegrass can have enhanced plant growth and tillering (Siegel et al. 1985; Latch 1994) and resistance to overgrazing by herbivores (Siegel et al. 1985; Familton et al. 1995). The presence of endophytes may inhibit the growth of companion legumes thereby reducing the overall productivity of pastures (Cosgrove 1993; Cunningham 1993a; Latch 1994). Perennial ryegrass containing *Neotyphodium lolii* is now generally preferred by the pastoral industry in New Zealand because it is more persistent under threat from drought and pasture pests such as Argentine stem weevil (Fletcher and Piper 1990). In New Zealand there is good evidence to show that perennial ryegrass will not persist in many districts if it is free from endophyte (Latch 1994).

However, some alkaloids produced from the association, when present in sufficiently high levels can adversely affect the health and production of grazing animals (Latch 1994). Effects on grazing animals include ryegrass staggers, reduced liveweight gains, increased faecal moisture (scouring) and faecal soiling (dags), increased incidence of

flystrike, increased rectal temperatures and respiration rates, and depressed serum prolactin levels (Fletcher 1993a; Familton et al. 1995). In New Zealand losses associated with *Neotyphodium lolii* infected ryegrass have been estimated to be in excess of \$100 million dollars (Familton et al. 1995). There is a complex matrix of interrelationships of different endophyte strains, ryegrass cultivars, alkaloids and animal responses (Fletcher 1993a; Familton et al. 1995). All the alkaloids produced by ryegrass/endophyte associations have a distinct seasonal profile with concentrations being highest in summer and autumn falling to minimal levels in winter and early spring (di Menna et al. 1992; Barker et al. 1993; Fletcher et al. 1996; Eerens et al. 1998a). Concentrations of metabolites are subject to wide variations due to seasonal and environmental factors (Powell et al. 1993b). Alkaloid production tended to increase when plants were water-stressed and heat-stressed (Eerens et al. 1998c) and the temperature under which plants are grown has been found to influence the production of lolitrem B and peramine (Barker et al. 1993; Lane et al. 1997).

2.4.1 Evaluation of ryegrass cultivars with endophyte

Endophytes can have an impact in breeding programmes, as the presence of endophytes can lead to misinterpretation of plant evaluation data because it increases biotic and abiotic host plant tolerance and causes toxicosis on grazing animals (Ravel et al. 1997). Differences in production due to endophyte highlight the need for caution in interpreting results from experiments when the endophyte status is unknown (Fletcher 1986). Field evaluation of new ryegrass/endophyte associations in different localities is necessary to determine the host benefits and the effects on the grazing animal (Siegel 1993).

2.4.2 *Neotyphodium lolii*

Neill (1940) showed that an endophytic fungus was normally present in the aerial parts of perennial ryegrasses in New Zealand. He found it to be concentrated in the leaf sheaths, flowering stems and seeds. The endophyte mycellium lives entirely within the intercellular spaces of the host tissue (Fletcher et al. 1990) and is not spread from plant to plant but is entirely seed-borne. Under the microscope endophyte can be found by

examination of stained leaf sheaths in which it appears as septate, rarely branched, parallel strands running the length of the sheath. A method of measuring the comparative amounts of fungus within infected plants uses a count of the number of strands seen per mm breadth of sheath. This count varies with season, reaching a peak in February and a low in August (Mortimer et al. 1984). The viability of endophyte mycelium in seed declines as the seed ages. The endophyte in perennial ryegrass seed will die out unless the seed is stored under conditions of low temperature and/or humidity (Fletcher et al. 1996). Most endophyte-infected seed that has been stored in seed warehouses for 2 years contains little or no viable endophyte (Siegel et al. 1985). Plants originating from stored seed are often endophyte-free depending on the age of the seed and the previous storage conditions (Fletcher et al. 1996).

Past breeding programmes for perennial ryegrass have emphasised increased persistence and production especially over summer. Fletcher et al. (1996) believed that these selection criteria has unwittingly favored endophyte infected plants, and 'prior to 1980 much of the so-called genetic improvement, recorded as a yield improvement in ryegrass cultivars in New Zealand may have been due to the presence of endophyte'. Once trading and marketing of new cultivars became established, there was an increase in the amount of seed stored to meet future demands. This led to an increase in endophyte-free seed. As a result of storing seed for varying lengths of time perennial ryegrass had a range of endophyte levels (Fletcher et al. 1996). After Prestidge et al. (1982) linked the persistence of ryegrass to endophyte, plant breeders began actively selecting for endophyte-infected ryegrass. Although there are now endophyte-free options, the greatest quantity of perennial ryegrass seed sold still has a high endophyte content (Fletcher et al. 1996).

2.4.3 Stem weevil resistance and peramine

The endophyte produces an alkaloid peramine, which deters the adult weevil from laying eggs in infected plants and can affect growth of the larvae (Rowan and Gaynor 1986; Tapper et al. 1989; Latch 1994). Peramine is thought to deter feeding of Argentine stem weevil adults and larvae at levels of 10 ppm (Rowan et al. 1990a).

Peramine concentrations in endophyte-infected ryegrass plants are variable with concentrations between 10 and 30 ppm being typical (Tapper et al. 1989). Peramine concentrations in the field are highest over summer when temperature is highest and rainfall lowest (di Menna et al. 1992; Eerens et al. 1998a). Peramine is relatively evenly distributed throughout the plant with concentrations being higher in younger leaves than in older leaves (Davies et al. 1993; Keogh 1993; Rowan 1993). It is apparent that peramine may be mobile within the ryegrass tiller (Keogh et al. 1996).

The main factors influencing changes in proportion of endophyte-infected tillers in a pasture is the severity of damage by Argentine stem weevil that preferentially attack endophyte-free tillers and climate. High summer temperatures and moisture stress can adversely affect the ability of the ryegrass plant to compensate for tiller losses due to Argentine stem weevil attack (Hume and Brock 1997).

2.4.4 Ryegrass staggers and lolitrem B

Symptoms, predisposing factors and causal alkaloids

The most visible effect of *Neotyphodium lolii* endophyte on animals is ryegrass staggers, named because of the staggering gait of affected animals, together with the observation that outbreaks tended to be on ryegrass-dominant pastures (Byford 1978). There is no relationship between ryegrass staggers and grass staggers (hypomagnesmia or grass tetany) which is a magnesium deficiency occurring mainly in dairy cows (Mortimer et al. 1982; Everest 1983).

Mortimer (1983) described the disorder as follows: 'As the neuromuscular disorder progresses there is head nodding and jerky movements. Interference with postural reflexes follows, seen as swaying or staggering during movement. As the condition worsens a stiff-legged gait may develop with short prancing steps usually resulting in arched back and rigid extended limbs held in a tetanic spasm of several minutes duration. This is followed by sudden muscular relaxation and apparent recovery, the animal then slowly regains its feet and walks away, often still showing tremors but with

very little locomotory inco-ordination. Cattle usually collapse onto the brisket with legs splayed, but generally remain upright either on their brisket or in a dog-sitting attitude'.

The severity of inco-ordination usually worsens the longer livestock are grazed on toxic pasture (Mortimer et al. 1984). Sheep ingesting toxic ryegrass material require 10-14 days for toxin levels to reach a threshold for significant ryegrass staggers to develop (Fletcher 1982). Forced exercise exacerbates the symptoms and is used to demonstrate the degree of incapacity present when scoring clinical severity in perennial ryegrass staggers in field trials (Keogh 1973). Symptoms of staggers appearing during this period are scored on a 0-5 scale for each animal (Appendix 2.1). Remission occurs spontaneously once good growth of pasture occurs or once animals are moved from toxic pasture (Rowan 1993) and stock usually make full recovery in 1 to 3 weeks (Mortimer et al. 1984).

Histological assessment of sheep severely affected by staggers has shown considerable damage to the brain (especially cerebellum) and spinal cord, but in most cases the damage could not be seen 3 weeks after recovery (Foot et al. 1987). There is other evidence suggesting lesions on autopsy are minimal (Mortimer 1983). Some farmers have observed apparent 'long term' effects in animals that have suffered severe ryegrass staggers (Foot et al. 1987). The enzymes aspartate transaminase and creatinine kinase may be potential indicators of the severity of ryegrass staggers (Piper 1989; Bray 1993; Fletcher 1993a). Ewes grazing high endophyte ryegrass have also shown significant differences in immune response to mitogens (Mc Farlane et al. 1993).

Staggers causes disruptions in management routines such as drenching and crutching, and can coincide with flushing and mating of ewes (Prestidge 1993). 'When feed is short the disease assumes a much more sinister form, production losses become severe and fatalities can be high' (Mortimer et al. 1982). Mortality is often caused by misadventure, such as drowning in streams after falling and becoming cast under fences (Prestidge 1993). Other stock losses occur through strangulation in fences and misadventure (Everest 1983). Collapsed animals can be attacked by seagulls and killed (Everest 1983). Difficult topography, poor access to drinking water and some farm

management practices exacerbate these problems (Prestidge 1993). The cost to the farmer can be 2-10% deaths, additional supplementary feedings, loss of stock condition and the labour requirement for increased stock surveillance (Everest 1983).

In a flock or herd there is a wide range of individual susceptibility to ryegrass staggers, often ranging from animals showing no obvious clinical symptoms to those unable to stand (Fletcher et al. 1990). Responses of individual animals to injections of lolitrem B varied widely eg. for lolitrem B dosed at 0.1 mg/kg, tremor scores ranged from 0 to almost 4 (Hawkes et al. 1993, 1995a and b). There is a strong heritable base to staggers susceptibility and major flock divergence in staggers can result from genetic selection (Hewett 1983; Morris et al. 1995). Cambell (1986) calculated a heritability of 0.47 ± 0.30 for ryegrass staggers resistance in Romneys.

Staggers is most prevalent in warm, drought-prone regions over dry summer-autumn conditions when stock closely graze ryegrass-dominant pastures containing *N.lolii* and a significant amount of dead and senescing plant material (Mortimer 1983; Fletcher and Piper 1990; Fletcher et al. 1990). Close grazing is implicated as a causal factor because *Neotyphodium lolii* and lolitrem B are concentrated in the leaf sheath in the vegetative plant tissue, with negligible amounts in the leaf (Fletcher and Piper 1990). Dead ryegrass tissue, whether generated by herbicide or natural senescence, increases the incidence and severity of staggers (Fletcher and Piper 1990). The disorder rarely occurs on irrigated pastures which characteristically have low dead matter content, high concentrations of *Neotyphodium lolii* and close grazing (Fletcher and Piper 1990). Ryegrass staggers never occurs in the winter and early spring despite a high dead matter content and extreme close grazing of high endophyte ryegrass (Fletcher and Piper 1990; Fletcher et al. 1990). Staggers is relatively uncommon in dairy cattle because animals are generally moved to fresh leafy pasture every 24 hours or less (Prestidge 1993).

From a farm survey in 1983 the factors causing staggers were found to be the proportion of ryegrass in the pasture, the height of the pasture and the age, condition, and species of the grazing animal (Harvey 1983). In a farm survey conducted in Australia in 1997 the majority of outbreaks of staggers were found in the autumn after a dry spell when

only short pasture was available (Wheatley 1997). In the farms surveyed staggers had occurred only once on 42% of farms, spasmodically on 17% of farms, every 4-5 years on 33% of farms and occurred every autumn on 8% of farms. Stock losses occurred on 33% of farms, were rare on 25% of farms and were not experienced on 42% of farms. Wheatley found that 91% of producers had never heard of endophyte or its associations.

Latch et al. (1976) and di Menna and Mantle (1976) postulated that a mycotoxin may be the cause of ryegrass staggers. A year later Gallagher et al. (1977) were able to show that fungal tremorgens can produce in sheep, signs, closely resembling those of ryegrass staggers when they were fed fungal spores from toxic pastures. A major advance into understanding the cause of staggers was the occurrence of a severe outbreak that disrupted a multi-replicated lamb grazing experiment comparing ryegrass cultivars (Fletcher and Harvey 1981; Fletcher 1982; Fletcher 1983). Grasslands 'Ruanui' which induced no ryegrass staggers symptoms in grazing hoggets was found to be completely free of endophyte. Hoggets grazing Grasslands 'Nui' and an old pasture line showed staggers symptoms. A firm association was found between the occurrence of staggers on the plots and the presence of endophyte in the ryegrass plants. Gallagher et al. (1981) reported the isolation of lolitrem A and lolitrem B neurotoxins and demonstrated tremorgenic activity in mice and then in sheep (1982). Mortimer and di Menna (1983) also demonstrated the correlation between endophyte and ryegrass staggers, and also to Argentine stem weevil resistance. Gallagher et al. (1984, 1985) reported the structure of the major neurotoxin, lolitrem B using a mouse behavioural bioassay to measure the tremorgenic activity of endophyte-infected pasture.

Lolitrem B is not uniformly distributed throughout vegetative ryegrass, but is present in higher concentrations in leaf sheath than in leaf blade (di Menna et al. 1992; Davies et al. 1993; Keogh and Tapper 1993; Keogh et al. 1996). Lolitrem B concentration increases progressively with increase in leaf age or maturity, reflecting the pattern and stage of development of both fungal and plant tissues (Keogh et al. 1996). Concentrations in flowering stems and seeds are also higher than in leaf blades (di Menna et al. 1992). A concentration of 2 ppm of lolitrem B is required for the development of clinical ryegrass staggers (di Menna et al. 1992; Blythe et al. 1993).

Hawkes et al. (1995c) reported that lolitrem B concentration was correlated with temperature, and that 2 ppm was equaled or exceeded when maximum temperatures were 21-27 °C. Peak alkaloid concentration was preceded by a sustained period of elevated temperatures and a minimum rainfall (Hawkes et al. 1995c). Lolitrem B concentrations are usually less than 1 ppm in winter, increase in spring and fall in late autumn (di Menna et al. 1992). Elevated levels of lolitrem B have been reported for Nui ryegrass with wild-type endophyte association of 4.2 to 5.4 ppm in March (Davies et al. 1993). An extreme lolitrem B concentration of 31 ppm has been recorded in a pseudostem sample from perennial ryegrass infected with an unidentified endophyte grown in the greenhouse (Lane et al. 1997b).

There is insufficient evidence to show conclusively that lolitrem B is the sole or even the major tremorgen affecting grazing sheep and cattle (Tapper 1993). Tremorgenic alkaloids may act synergistically with other endophyte/ryegrass alkaloids in the incidence of staggers (Gallagher et al. 1977; Fletcher et al. 1993). Lolitrem B is believed to be the end product of the indole diterpenoid pathway in which paxilline, also a tremorgen, is a key intermediate (Garthwaite et al. 1993; Penn 1993; Rowan 1993). A number of other indole diterpenoids are also produced, some of which are toxic although not tremorgenic (Garthwaite et al. 1993). Fletcher et al. (1991) and Fletcher (1993) reported that lambs grazing one cultivar of perennial ryegrass infected with 187BB endophyte, a strain which does not produce lolitrem B, experienced mild staggers. These staggers were correlated with concentrations of paxilline. Cosgrove (1993) reported that the endophyte strain 187BB eliminated the incidence of ryegrass staggers in grazing dairy beef bulls.

Results from dosing lolitrem B and paxilline to sheep indicate that lolitrem B is approximately 10 times as tremorgenic as paxilline, and that it exerts its tremorgenic effect for 10-20 times longer than paxilline (Hawkes et al. 1993, 1995a; Miles et al. 1993b). Paxilline is rapidly eliminated from the body or metabolised to a non-tremorgenic form (Hawkes et al. 1995b). Miles et al. (1993b) reported that paxilline alone is very unlikely to cause tremors in sheep in the field. However, more than 10 compounds have been detected that are structurally related to paxilline, and there is a

possibility that some of these compounds, either alone or in combination, are sufficiently tremorgenic, or present in high enough quantities in ryegrass, to cause ryegrass staggers in sheep (Miles et al. 1993b). Paxilline-like compounds appear to be relatively evenly distributed throughout the plant (Davies et al. 1993). Davies et al. (1993) has reported elevated levels of paxilline-like compounds in Ruanui with 187BB endophyte in March (5.3 ppm) and for synthetic endophyte associations with ryegrass in April (4.1-6.0 ppm).

Management

The main factors contributing to an outbreak of ryegrass staggers are pasture composition and structure, environmental conditions, grazing management, and animal feeding behaviour, which determine the production, distribution and ingestion of tremorgen alkaloids (Keogh 1983). Development of staggers is dependent on the intake of sufficient causal toxins, which is a function of toxin distribution and livestock defoliation patterns (Keogh 1986). Any management strategy to minimise the toxic effects of ryegrass/endophyte associations on grazing animals must be centered on minimising the toxin intake of the grazing animal or toxin production by the ryegrass/endophyte association (Fletcher 1993a; Keogh and Clements 1993). Palatability is affected and when given the choice, animals prefer to eat endophyte-free grass. However, with increased grazing pressure animals are forced to consume leaf sheath and dead material, increasing their consumption of toxic material (Edwards et al. 1993; Latch 1994). The Code of Animal Welfare (Animal Welfare Advisory Committee 1996) specifies that sheep exhibiting staggers should be moved onto low risk pasture, low-endophyte ryegrass, endophyte-free ryegrass, onto a crop, or fed hay. Movement should be slow and animals left undisturbed as much as possible. Paddocks with natural hazards such as ponds, ditches and bluffs should be avoided.

Management strategies are as follows:

- 1) Staggers symptoms should be recognised early and affected animals moved to safe grazing or fed supplements.
- 2) In areas subject to chronic ryegrass staggers, farmers should consider setting aside an area specifically for relieving the effects of ryegrass staggers. This area should be sown with endophyte-free ryegrass or alternative grass, legume, or other forage species (Mortimer et al. 1982; Fletcher 1993a; Prestidge and Thom 1994).
- 3) Incorporate companion species for endophyte-infected perennial ryegrass such as white clover, in order to dilute the intake of the toxins (Fletcher 1993a; Prestidge 1993; Prestidge and Thom 1994). Control measures should include avoidance of practices leading to the development of ryegrass dominance during summer/autumn period (Keogh 1978).
- 4) Prevent close or overgrazing during periods when severe outbreaks are likely to occur in summer and autumn (Keogh 1973 1978; Mortimer et al. 1982).
- 5) Breeding for staggers-resistant animals (Prestidge 1993). It is possible to breed for increased tolerance to ryegrass staggers (Hewett 1983; Morris et al. 1995). Progress depends on availability of toxic pasture (Miles et al. 1993a). In addition the requirement of high grazing pressure to force the animals to graze toxic pasture can result in substantial losses in body condition (Hawkes et al. 1993; Morris et al. 1995).
- 6) Utilisation of low tremorgen-producing endophytes (Fletcher 1993a; Prestidge 1993).
- 7) Use rotational grazing with a rapid rotation with daily shifts to prevent regazing of previously grazed areas. Urine patches contain higher concentrations of endophyte (Keogh 1984). Set stocked lambs remove more leaf close to the ligule and can be

more affected by ryegrass staggers. Rotational grazing gives more control over the extent of defoliation on grass dominant pasture (Keogh and Clements 1993) and set stocking should be avoided (Mortimer et al. 1982). The production and accumulation of lolitrem B may be minimised if the ryegrass component of pastures is maintained at a young leafy stage and not allowed to be spelled for too long so that mature and senescent leaves predominate (Keogh and Tapper 1993).

- 8) Sow endophyte-free ryegrass. The health and performance of animals can be improved by sowing endophyte-free ryegrass, however it will not persist in many areas of New Zealand because of pests and drought (Fletcher and Easton 1997). Endophyte is not needed in cool-moist environments in Southern New Zealand (Eerens et al. 1997a; Eerens et al. 1998b).

Management strategies to minimise toxic effects in grazing animals are only an option if the farming system has the flexibility to practically incorporate such measures. For example, in drought prone areas where warm summer dry conditions lead to general feed shortages in late summer-autumn leading to overgrazing, the lower horizons of the plant lead to staggers and other toxicity problems (Familton et al. 1995). However, hard grazing is necessary at times (eg autumn clean-up of pasture) to remove the mature leaf and leaf litter which would otherwise accumulate and pose a greater risk if grazed when neurotoxin levels were at a peak (Keogh 1993; Keogh and Clements 1993). With continuing grass growth the animal's intake of fresh grass proportionally increases, and the incidence of staggers will diminish (Latch et al. 1976).

2.4.5 Heat stress and ergovaline

Tall fescue summer toxicosis

Endophyte *Neotyphodium coenophialum* in tall fescue (*Festuca arundinacea* Schreb.) in the USA produces summer toxicosis in beef cattle. This association can cause lower feed intake, lower weight gains, lower milk production, higher respiration rates, higher body temperatures, reduced serum prolactin levels and reduced reproductive performance (Bacon et al. 1986; Hoveland 1993; Ball 1997). Steers grazing tall fescue infected with

endophyte were less active and spent less time grazing than steers on tall fescue not infected with endophyte (Seman et al. 1990). The ergopeptine alkaloids have been implicated, and of these ergovaline occurs in the greatest concentration (Fletcher 1993b) and is one of the most toxic (Bacon et al. 1986). Feeding levels of endophyte-infected tall fescue seed with ergovaline levels 500 to 2000 ppb significantly reduced feed intake and average daily gain of lambs as compared to controls (Debassai 1993). The serum prolactin levels were markedly lowered and lambs on the ergovaline-containing diets were lethargic and depressed (Debassai 1993).

Symptoms, predisposing factors and causal alkaloids

Ergopeptine alkaloids also occur in perennial ryegrass (*Lolium perenne*) infected with the endophytic fungus *Neotyphodium lolii* (Rowan and Shaw 1987). Ergovaline has been detected in ryegrass infected with wild type endophytes at similar levels to those of tall fescue infected with *N. coenophialum* (Rowan et al. 1990b). Easton et al. (1995) believes that ergovaline levels in perennial ryegrass pastures are high enough to cause fescue toxicosis symptoms in livestock if ambient weather conditions are suitable. The ergopeptine alkaloids are dopamine agonists which can act as vaso-constrictors and reduce peripheral blood flow and the ability to dissipate excess heat (Rhodes et al. 1991). Latch (1994) believes that few farmers are aware of the effects that toxins such as ergovaline may have on animal production.

Sheep grazing endophyte infected ryegrass have reduced serum prolactin levels and liveweight gains (Fletcher and Barrell 1984), an effect possibly due to the presence of ergopeptine alkaloids (Rowan et al. 1990b). Heat stress problems were first exposed in experiments using endophyte-infected ryegrass pasture which did not produce lolitrem B (187BB endophyte) and where the usual symptoms of ryegrass staggers were not observed (Fletcher 1993b, Fletcher and Sutherland 1993). The highest rectal temperatures and respiration rates were in lambs grazing the ryegrass/endophyte associations producing the highest ergovaline levels (Fletcher 1993a). Respiratory problems such as excessive coughing and slow labored breathing were more evident in

lambs grazing ryegrass infected with 187BB endophyte which had the highest ergovaline concentrations.

Conclusive confirmation of ergovaline as the causative agent in this heat stress syndrome is not possible due to confounding with other endophyte/ryegrass alkaloids such as paxilline (Fletcher 1993b). Ergovaline is not believed to be linked to the ryegrass staggers syndrome, since administration of the dopamine antagonist metoclopramide restored prolactin to levels of sheep grazing endophyte free ryegrass but had no effect on ryegrass staggers (Piper and Fletcher 1990). It is now recognised that the dominant and debilitating effects of ryegrass staggers may have been obscuring more subtle symptoms of heat stress (Fletcher 1993b). Foot (1997) reported catastrophic events of ryegrass staggers in Australia resulting in large numbers of animals dying in creeks or dams, an effect possibly exacerbated by heat stress.

Observations of heat stress have been reported in cattle exhibiting decreased milk production, and depressed appetite. Cattle were visibly stressed, drooling and in some cases panting (Easton et al. 1995). The symptoms were similar to those described for tall fescue toxicosis (Easton et al. 1995). Easton et al. (1995) believes that it is not unusual for cattle grazing endophyte-infected ryegrass in northern New Zealand to ingest herbage containing over 1.0 ppm ergovaline, and sometimes over 1.5 ppm. Easton et al. (1995) believes that the relatively infrequent incidence of clinical heat stress in New Zealand cattle grazing perennial ryegrass is due to relatively short-term exposure to elevated levels of ergovaline, and to the lower ambient conditions compared to those of southeastern United States of America.

Grazing endophyte-infected ryegrass at ambient temperatures below 25 °C, may not create a heat stress problem but temperatures above this may result in a significant effect on animal production (Fletcher 1993a; Familton et al. 1995). The critical ambient temperature above which hyperthermia was evoked in rats by ergotamine (structurally similar to ergovaline) dosing was 30 °C and an interaction between dose rate and environmental temperature has been observed (Smith et al. 1993; Smith et al. 1995). Reducing intake is a normal thermo-regulatory response to hyperthermia. These effects

are likely to be exaggerated in large animals such as dairy cows with a high metabolic rate and a higher rate of excess heat production (Fletcher and Easton 1997). It is thought that sheep are more tolerant of high ambient temperatures (Fletcher 1993a).

Comprehensive studies in several trials, covering a range of conditions, have revealed that reduced prolactin levels are one of the most consistent responses to grazing ryegrass endophyte associations and associated ergovaline levels (Fletcher et al. 1996). Prolactin may also be an important link in mediating, at least in part, some of the effects of ergopeptine alkaloids or ergovaline on animal responses (Fletcher et al. 1996). Prolactin levels in healthy sheep tend to increase as photoperiod, ambient temperature and consequent heat load on the animal increase (Fletcher et al. 1997). As ambient temperature increases, prolactin levels in sheep grazing endophyte-free grasses increases rapidly, while those grazing endophyte associations with ergovaline fail to respond (Fletcher and Easton 1997). The prolactin response is characterised by a low threshold to ergovaline, with maximum depression occurring at or below 0.5 ppm ergovaline in pastures (Cheeke, et al. 1993). Significantly reduced serum prolactin levels in sheep grazing endophyte have been reported (Fletcher and Barell 1984; Fletcher 1993b). Fletcher et al. (1996) reported prolactin levels in ewes of 175 ng/ml for endophyte-free ryegrass, and 74 ng/ml for wild-type endophyte-infected ryegrass, taken during February.

Significant increases in body temperature and respiration rate of lambs grazing endophyte-infected pastures have been reported (Fletcher et al. 1994; Fletcher and Easton 1997). Body temperatures of grazing lambs, measured on six dates during summer, were 40.4, 40.9 and 40.85 °C respectively for lambs grazing endophyte-free ryegrass, ryegrass with its wild-type endophyte and ryegrass infected with 187BB endophyte (Fletcher and Easton 1997). In a number of field trials at Lincoln, maximum body temperature was reached at an ergovaline concentration of 1.0 ppm ergovaline under warm field conditions (Fletcher and Easton 1997).

Ergovaline concentrations are highest in the tissues most important for the survival and dissemination of the endophyte, namely the crown, the source of new emerging tillers,

and the developing reproductive organs (Davies et al. 1993; Easton et al. 1993; Lane et al. 1997a). Ergovaline levels vary through the season, between districts and according to management (Easton et al. 1993). Measured ergovaline concentrations are evidently highly sensitive to experimental conditions (Davies et al. 1993). There is a wide variation in ergovaline concentration between individual plants (Rowan et al. 1990b; Lane et al. 1997a). Concentrations have been found to increase when plants are under water deficit conditions (Barker et al. 1993; Lane et al. 1997c). Typically in pastures, concentrations are lowest in winter (Rowan et al. 1990b) with an initial peak in December with reproductive development, and another peak in late summer associated with a low pasture profile and heat stress (Easton et al. 1993). Ergovaline levels of 0.5-1.0 ppm are commonplace on leafy pasture of commonly sown cultivars (Easton et al. 1993; Davies et al. 1993; Woodburn 1993;). These values are comparable to concentrations reported for tall fescue herbage in the United States (Rowan et al. 1990b). Lane et al. (1997b) has found extreme ergovaline concentrations exceeding 15 ppm in perennial ryegrass with natural endophyte and unidentified strains from greenhouse-grown pseudostem fractions.

Management

Ryegrass hay or straw should be analysed for ergovaline concentration to determine the risk of feeding it (Welty 1993). Grazing management systems to minimise grazing basal parts of the ryegrass plant where ergovaline is concentrated has yet to be experimentally tested as a means of minimising heat stress in ryegrass/endophyte associations (Familton et al. 1995).

2.4.6 Faecal contamination

Faecal contamination (dags) and ovine myiasis (flystrike) are of major economic importance to the sheep industry of New Zealand. Flystrike can cause loss of liveweight, deaths and wool and pelt damage, and requires application of insecticides for its control. Fletcher and Sutherland (1993a) suggest that at least some of the recent increase in faecal moisture (scouring), dags and flystrike since 1981, may be linked to

an increase in the area of high endophyte ryegrass under grazing. Lambs grazing endophyte-infected ryegrass over summer and autumn have consistently had increased faecal moisture equating to a significant increase in observed scouring in lambs (Eerens 1992; Fletcher and Sutherland 1993a; Fletcher et al. 1996). The toxic agent responsible appears most likely to be one of the mobile alkaloids concentrated in the leaf or distributed throughout the plant (Pownall et al. 1993a and b; Tapper 1993). Pownall et al. (1993a and b) concluded that metabolites other than lolitrem B were responsible for increased faecal moisture and dags, and that of the measured alkaloids only peramine appeared to be statistically linked to faecal dry matter. However, when synthetic peramine was administered to wether lambs there was no effect on faecal moisture (Pownall et al. 1995). Paxilline and other unidentified alkaloids may be involved, but it has not been possible to make a definitive link with any one toxin(s) (Fletcher 1993a).

2.4.7 Liveweight gain

Before 1981 farmers reported summer-autumn ill thrift syndrome in sheep and cattle, which was characterised by negligible weight gains over variable periods in summer and autumn. Summer-autumn ill thrift syndrome may have been caused through grazing endophyte-infected pastures (Fletcher et al. 1996). Endophyte in ryegrass has been shown to decrease liveweight gain, from late spring through to autumn (Fletcher 1983; Fletcher and Barrell 1984; Fletcher 1986; Fletcher et al. 1990, Eerens et al. 1992; Fletcher and Sutherland 1993b; Foot et al. 1997). Liveweight gains in lambs grazing endophyte-free ryegrass over summer and autumn have consistently been 100% higher than for those of lambs grazing the same ryegrass with its wild-type endophyte (102 g/day vs 36 g/day mean from several trials; Fletcher et al. 1996). Liveweight change reflects the dominant effects of endophyte and ryegrass cultivar which encompasses many interrelated and significant primary endophyte effects including dags, flystrike, ryegrass staggers, heat stress and alkaloid concentrations (Fletcher and Sutherland 1993, Fletcher 1993b, Fletcher et al. 1993, Davies et al. 1993). In the cases of severe ryegrass staggers, reduced intake may be due to impaired grazing ability and a primary toxic effect (Fletcher 1993a). It is unclear whether the dominant effect of endophyte on liveweight change is a direct toxic effect on the animal or a secondary effect of reduced

intake through lower palatability or reduced appetite via neuro-chemical effects (Fletcher 1993a).

Liveweight gains may be reduced on endophyte infected ryegrass in the absence of clinical ryegrass staggers (Fletcher and Barell 1984; Fletcher and Piper 1990; Eerens 1992). Fletcher and Sutherland (1993b) reported that liveweight gains in lambs grazing ryegrass infected with the 187BB endophyte were intermediate between those grazing the same ryegrass with wild-type endophyte and endophyte-free ryegrass. The poorer liveweight gains in lambs grazing Nui with wild-type endophyte was attributed to serious ryegrass staggers (Fletcher et al. 1993). However, over an extended grazing period there was no significant difference in liveweight gain between Nui with wild-type endophyte and both Nui and Ruanui cultivars with 187BB endophyte, so other toxins appeared to be exerting a greater effect on liveweight gain (Fletcher and Sutherland 1993b).

2.4.8 Milk production and reproduction

In an Australian study, Valentine et al. (1993) showed that cows grazing high endophyte ryegrass had reduced milk production (up to 2.2 litres/cow/day) and milk protein yield during March. However, results from this study can not be extrapolated to New Zealand conditions because cows were grazing irrigated pure ryegrass swards in a warm/hot climate (Prestidge and Thom 1994). Clark et al. (1996) reported that milk yield from cows fed the low endophyte pasture and silage were 10.6% higher than cows fed high endophyte silage on high endophyte pastures, and related the better performance to a lower intake of ergovaline. In an extensive farmlet trial in the Waikato carried over three years it was found that endophyte caused small (5-6%), inconsistent (2 out of 9 test periods) reductions in milk production. These effects were not strongly related to peak levels of lolitrem B in the ryegrass or to the incidence of ryegrass staggers. Rectal temperatures did not indicate cows were heat stressed. Ergovaline levels were less than 1 ppm and ambient temperatures rarely exceeded 25 °C. (Thom et al. 1994; Thom 1997, Thom et al. 1997a and b). Cosgrove (1996)

reported that the effects of endophyte alkaloids on liveweight gain of dairy beef cattle were small and influenced by season.

Ingestion of endophyte-infected ryegrasses has been found to reduce plasma testosterone levels in rams and bulls, and reduced sperm counts in rams (Prestidge 1993). Foot et al. (1988) reported lamb mortality was two to three times higher on high-endophyte plots than on the equivalent low-endophyte plots. Eerens et al. (1994, 1997a and b) found that endophyte infected pastures delayed lamb drop by an average of 3-5 days or 0.68 days in synchronised ewes, and reduced lamb liveweight gains during the lactation period in Southland. The poor lamb performance in these experiments was attributed to ewe exposure to high endophyte pasture in the previous summer. Endophyte had no effect on lamb birth weight or ewe conception rate and liveweight changes over autumn, winter and spring (Eerens et al. 1994; 1997a).

2.4.9 Novel endophyte associations

Novel associations of ryegrass and endophyte genotypes can be established by inoculating endophyte-free ryegrasses with mycelium from isolated endophytes (Latch and Christensen 1985). The identification and selection of an endophyte which does not produce lolitrem B but does produce the insect-deterrent peramine (187BB/endosafe) in association with its host ryegrass, was a major breakthrough in practical control of ryegrass staggers (Latch and Christensen 1985). Two cultivars were infected with 187BB endophyte and field-testing showed that these grasses were protected from weevil predation and sheep grazing them did not develop typical ryegrass staggers (Fletcher et al. 1991; Fletcher et al. 1993). Similar results were obtained with grazing bulls (Cosgrove et al. 1993). However, further testing after they were released indicated that the level of ergovaline in the cultivar Pacific infected with 187BB endophyte was higher than in many other endophyte infected ryegrasses and this material was withdrawn from the market. The second cultivar, Greenstone which is a tetraploid hybrid ryegrass had a much lower level of ergovaline and it is still being marketed successfully (Latch 1994). Further strains of endophytes from ryegrass have been identified which produce neither lolitrem B nor ergovaline and these strains have been

artificially infected into several cultivars of perennial ryegrass. Animal feeding trials will commence shortly with these strains to determine whether they are an improvement on strain 187BB (Latch and Fletcher 1997).

2.4.10 Effect of host cultivar on alkaloid production

The introduction of selected endophyte strains into different ryegrass cultivars has exposed cultivar or plant genotype effects on several ryegrass/endophyte alkaloids and consequent animal responses, which in some cases had previously been obscured by ryegrass staggers (Fletcher and Sutherland 1993a). The strain of endophyte and the plant host genotype can determine the presence and quantity of the alkaloids lolitrem B, peramine and ergovaline produced and animal response (Barker et al. 1993; Davies et al. 1993; Garthwaite et al. 1993; Fletcher and Sutherland 1993a; Powell et al. 1993b; Latch 1997). Each grass-fungal pair results in a unique combination of alkaloids and other secondary metabolites (Powell et al. 1993b). Alkaloid profiles may be useful as “fingerprints” for identification of specific grass/endophyte pairs (Powell 1993a). Davies et al. (1993) demonstrated the effects of endophyte strain and plant genotype on alkaloid production. The different ryegrass/endophyte associations produced distinctly different alkaloid profiles (Table 2.3 and 2.4).

The modifying effect of the host ryegrass on alkaloid production and consequent animal response is unexplained but could be due to reduced concentrations of endophyte mycelium within the plant (Fletcher 1993a), differences in vigor of endophyte growth in the plant or to a more specific regulatory effect of the plant on endophyte alkaloid metabolism (Davies et al. 1993). A modifying effect of plant genotype has also been noted in tall fescue (Hill 1993). The interaction between host plant genotype and endophyte may vary with different environmental conditions (Fletcher et al. 1991).

Differences between cultivars in their production of alkaloids when infected with the same strain of endophyte pose difficulties in selecting strains for general use (Latch 1994). Differences in alkaloid production also occur between plants in the same cultivar. For example concentrations of ergovaline in leaf sheath varied from 2.5 to

27.2 ppm in 19 seedlings of Grassland Nui (Latch 1994). Therefore it is desirable that strains being considered for general use should not have the capability of producing the particular toxic compound one is concerned about. Therefore endophyte strains need to be tested on a wide range of plant genotypes and only those strains found incapable of producing undesirable toxins should be considered for future studies (Latch 1994).

Table 2.3 Concentrations (ppm) of alkaloids produced by **Nui** perennial ryegrass with different endophyte genotypes. Mean of March and April harvests to ground level. Adapted from Davies et al. (1993).

Endophyte genotype	Lolitrein B	Ergovaline	Peramine	Paxilline-like compounds
Wild-type	4.0	1.3	20.4	3.5
196	0.9	4.1	22.1	5.0
187BB	0.6	4.1	24.0	5.0

Table 2.4 Concentrations (ppm) of alkaloids produced by **187BB** endophyte with different ryegrass cultivars. Mean of March and April harvests. Adapted from Davies et al. (1993).

Plant cultivar	Lolitrein B	Ergovaline	Peramine	Paxilline-like compounds
Nui	0.6	4.1	24.0	5.0
Ruanui	0	4.7	25.9	5.7
Greenstone	0	1.3	16.3	2.8

2.4.11 Future developments

New strains of endophyte

Endophytes, which do not produce lolitrem B or ergovaline have now been isolated and inoculated into perennial ryegrass. The development of these 'non toxic' endophytes with selected alkaloid profiles have the potential to substantially improve animal health and performance from persistent ryegrasses in the future (Fletcher et al. 1994; Fletcher et al. 1996). These associations will undergo rigorous testing before release to the industry (Fletcher and Easton 1997).

Other

Possible future strategies include developing antidotes and immunisation against toxic alkaloids for grazing animals (Cunningham et al. 1993a; Siegel 1993; Fletcher et al. 1996) and manipulation of rumen microflora for detoxification of alkaloids. There is also potential for genetically modified endophytes which could produce a range of insecticidal, fungicidal and antibiotic compounds, and development of genetic resistance in livestock (Fletcher et al. 1996; Fletcher and Easton 1997). Significant control of Argentine stem weevil by a recently released parasitoid wasp *Microctonus hyperodae* may reduce the need for high levels of insect deterrents (Fletcher and Easton 1997).

Prediction

The development of the ELISA system for estimating lolitrem B concentration could be a useful management tool in helping to identify danger periods when the risks of outbreaks of ryegrass staggers occurring in livestock grazing ryegrass-based pastures are high (Keogh 1996). It may also be possible to predict the relative neurotoxicity levels within and between ryegrass-based pastures using information on *Neotyphodium lolii* concentrations (Keogh 1996). Weather parameters could be recorded on farms for

the purpose of predicting ryegrass stagger danger periods, thereby allowing stock and pasture management practices to minimise staggers incidence (Hawkes et al. 1995c).

2.5 CONCLUSIONS

Much of the genetic variation in organic matter digestibility observed within perennial ryegrass is due to differences in maturity or earliness of flowering. Improvement in digestibility by breeding can potentially operate through a number of indirect pathways including later flowering date, greater leafiness, reduced lignification or high proportion of cell contents, or directly through selection for whole plant digestibility. There is disagreement in the literature on the progress achieved to date in developing cultivars with improved nutritive value. The adoption of new cultivars by New Zealand farmers is low. Many farmers believe that improved management of existing pasture will generate greater returns than introducing new cultivars. Nevertheless, Davies et al. (1993) demonstrated significant differences in sheep production in a six-year evaluation of perennial ryegrass cultivars under continuous stocking management, where sheep production was positively correlated to organic matter digestibility.

The ultimate test of a new cultivar should be its performance in a production system. It is strongly recommended that new cultivars be evaluated early in the breeding process using animal production trials. Attempting to breed forages for improved forage quality without conducting animal evaluation trials can lead to erroneous conclusions (Vogel and Sleper 1994). Detailed measurements are required to understand observed levels of animal production, and reasons for variation between years. The main source of error in the measurement of animal production is the variability between animals. Compatibility with companion clover, and endophyte alkaloid effects, should also be examined.

The perception of genetic differences between cultivars in the past has been impaired by the presence of endophyte. Endophyte in ryegrass can reduce liveweight gain from late spring through to autumn, reflecting the interrelated effects of alkaloid concentrations, ryegrass staggers, heat stress and faecal contamination. Each endophyte/cultivar combination produces a unique combination of alkaloid and animal responses. The

development of non-toxic endophytes offers the potential to improve animal health and performance from ryegrasses while retaining insect resistance in the future.

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CHAPTER 3

EXPERIMENT 1: ANIMAL EVALUATION OF ARIES HD PERENNIAL RYEGRASS SELECTED FOR HIGH DIGESTIBILITY

3.1 ABSTRACT

Aries HD perennial ryegrass (*Lolium perenne* L.) has been selected for increased digestibility in summer and early autumn. It is claimed to be the first commercial perennial ryegrass selected specifically for improved digestibility. The relative performance of weaned Romney lambs over summer was compared using Yatsyn 1 perennial ryegrass as a control cultivar. There were three replicates of each cultivar of 0.33 ha initially stocked with 8 lambs/plot (24 lambs/ha). Swards were continuously stocked with lambs with a variable stocking rate to maintain a mean sward surface height of 6 cm. Lambs grazing Aries HD plots consistently achieved higher liveweight gains over the experiment. Liveweight gain was higher on Aries HD than Yatsyn 1 during summer dry conditions 24/1/96 to 4/3/96 (50 vs 31 ± 7.8 g/day, $P < 0.035$) and overall (104 vs 84 ± 4.6 g/day, $P < 0.103$). During summer on average 15% of lambs grazing Aries HD pasture were severely affected by perennial ryegrass staggers compared with 29% of lambs on Yatsyn 1 pastures ($P < 0.027$). Information on pasture quality, alkaloid concentrations, and herbage intake is presented and discussed in relation to the differences in animal performance.

This chapter forms the basis of a paper submitted to: *The Proceedings of the New Zealand Grassland Association* 59: 245-249 (Appendix 3.1).

3.2 INTRODUCTION

To date there has been a lack of breeding progress for improved forage digestibility, although genetic variation for *in vitro* digestibility has been reported. Past breeding programmes emphasised other traits because the economic value of breeding for improved digestibility was not recognised, or other traits were considered to be more important (Vogel and Sleper 1994). However, the cultivars that have been developed and released with improved forage quality have demonstrated a greater improvement in profitability than those offering similar increases in forage yield. Examples are selections in bermudagrass, switchgrass, wheatgrass and lucerne (Vogel and Sleper 1994).

Gains from improved digestibility must be validated in animal experiments (Vogel and Sleper 1994). Animal production experiments are the most direct means of detecting promising cultivars, assessing nutritive value continuously, providing economic assessment and testing persistence under commercial conditions (Laidlaw and Reed 1993). They also provide an opportunity to detect adverse effects from the perennial ryegrass association with endophyte (*Neotyphodium lolii*). In addition they play a role in technology transfer by providing relevant information for farmers.

Wrightson Seeds Ltd selected Aries HD perennial ryegrass for improved forage quality over the crucial summer/autumn period. They have demonstrated organic matter digestibilities of 87.2% for Aries HD vs 84.6% for Yatsyn 1 (Appendix 1.1) under rotational cutting management at Kimihia Research Centre in Christchurch. The aim of the current experiment was to evaluate whether this small demonstrated increase in digestibility could be translated into greater animal production.

3.3 MATERIALS AND METHODS

Clover-free swards of Aries HD and Yatsyn 1 were established on the Sheep and Beef Cattle Research Area, Massey University in the autumn of 1995, in a randomised

complete block design (Appendix 3.2) with three blocks (0.33 ha/plot). The summer lamb finishing experiment began 11 December 1995 and concluded 25 April 1996.

A total of 58 Romney weaned wether lambs were selected for the experiment in late November 1995. Ten lambs were allocated to an initial slaughter group and their carcass weights were used to predict initial carcass weight of the remaining 48 lambs at the start of the experiment. These lambs were allocated to plots on 11 December in a stratified manner according to liveweight. Lambs were drenched monthly with Ivomec (ivomectin; Merk, Sharp and Dohme, NZ Ltd) to control internal parasites, and dipped with Vetrazin (cyromazine; Ciba-Geigy, NZ Ltd) in January to prevent fly strike.

Plots were grazed continuously to a sward surface height of 6 cm to ensure optimum feed intake and equal herbage allowance between plots (Hodgson 1990). This height was monitored twice per week with a sward stick, and maintained by adjusting lamb numbers. There were regular monthly applications of urea or Cropmaster 15 (Ravensdown Fertiliser Co-operative Ltd, New Zealand), equivalent to a total of 147 kg N/ha, 27.5 kg P/ha, 27.5 kg K/ha and 20.8 kg S/ha over the experiment. A sprinkler irrigation system was used in March during a severe dry spell. There were two applications of Versatill (clopyralid amine; DowElanco, NZ Ltd) at 1.5 litres/ha to eliminate weeds and volunteer clover in the pasture. A general view of the experimental site is shown in Plate 3.1.

Herbage mass was determined fortnightly by cutting six 0.1 m² quadrats per plot to ground level. Botanical composition was also determined on bulked ground level samples from each plot. Pasture cuts to ground level and hand plucked samples were taken fortnightly for analysis of *in vitro* organic matter digestibility (Roughan and Holland 1977), nitrogen (Kjeldahl procedure) and neutral detergent fibre content (Van Soest 1994). Monthly ground level samples were also analysed for concentrations of lolitrem B, peramine and ergovaline by HPLC (Baker *et al.* 1993).

Plate 3.1 General view of experimental swards in Experiment 1.



Plate 3.2 Lambs after shearing at the end of Experiment 1.



Lamb unfasted liveweight was recorded fortnightly. All lambs were slaughtered on 30 April 1996 and carcass weights and fat depth (GR) were recorded. Lambs were shorn one week prior to slaughter (Plate 3.1) and greasy wool weighed. The wool yield was determined (clean weight/greasy weight) as described by Min et al. (1998). Incidence of severe ryegrass staggers was recorded as the number of lambs carried to weighing each fortnight and was analysed using a chi-square test adjusted using a Yates correction factor (Little and Hills 1978).

Herbage intake was measured from 19 to 25 April 1996 using intra-ruminal slow release chromium sesquioxide capsules (Captech New Zealand Ltd) as described by Parker *et al.* (1989). Faecal grab samples were oven dried, bulked across days on an equal dry weight basis and ground. Two sheep fistulated in the oesophagus were rotated among the swards, to collect samples of the herbage selected for calculation of organic matter digestibility. Chromium release rate was estimated by recovering capsules at slaughter.

Observations of daylight grazing behaviour were made from a van parked in the plots. Grazing activities were recorded at intervals of 10 minutes during one continuous period of 12 hours twice during the intake period. Observations were recorded for the number of lambs grazing, ruminating or idling in each plot. Bite rates (time taken for 20 bites) were recorded on random animals in each plot during the periods between grazing observations (Jamieson and Hodgson 1979).

Analysis of variance and repeated measures analysis were carried out using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc. 1988). All statistical analyses were based on plot mean values with three replicates. Individual animal performance was calculated using data from core group animals and least squares means analysis reported where numbers were unbalanced.

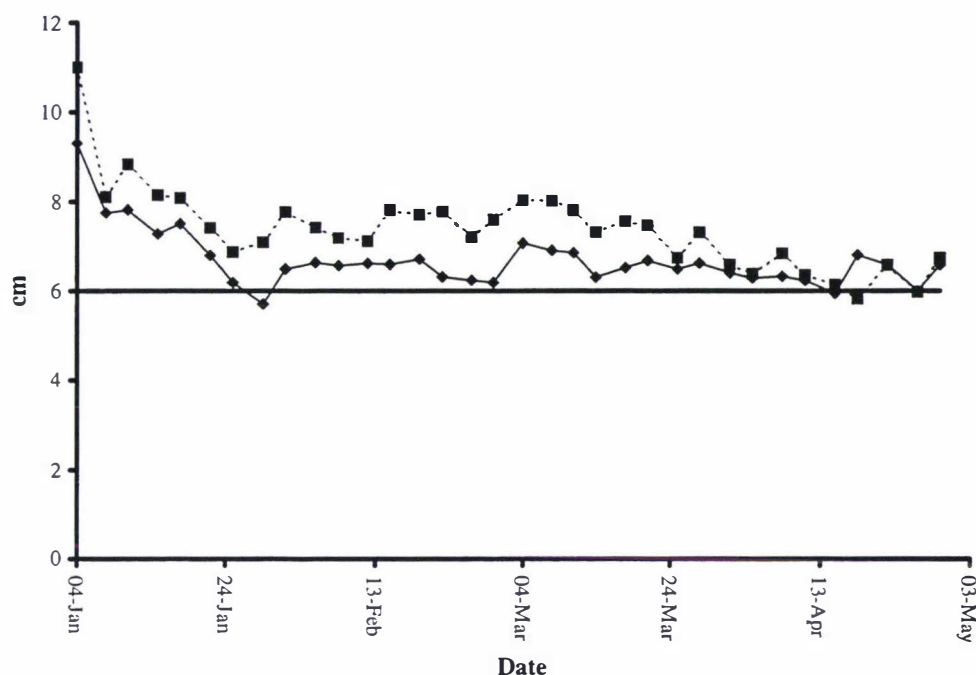
3.4 RESULTS

Weather conditions over the experiment are included in Appendix 3.3. Mean herbage mass and sward height was similar over the experiment for the two cultivars (Table 3.1 and Figure 3.1). Lamb stocking rate did not differ significantly between cultivars (Table 3.2). For both cultivars ryegrass was the principal component of the pasture on offer (over 80% live herbage). Clover content in the sward was negligible. The proportion of tillers infected with endophyte was over 90% in both cultivars.

Table 3.1 Cultivar effects on mean herbage mass, sward height, botanical composition and the proportion of tillers infected with endophyte.

Sward Measurements	Aries HD	Yatsyn 1	SEM	P
Herbage mass (kg DM/ha)	2865	3108	517.6	0.3847
Sward height (cm) 4/1/96 - 29/4/96	6.68	7.37	1.391	0.1658
% Ryegrass in live herbage	81.9	86.4	7.73	0.3030
% Leaf in ryegrass	71.8	70.9	1.27	0.4065
% Endophyte infection on 14/5/96	90.8	91.7	3.58	0.8845

Figure 3.1 Mean sward height for Aries HD (◆) and Yatsyn 1 (■) pasture and 6-cm target line.



Over the entire experiment Aries HD lambs gained 20 g/day (23% advantage) more than Yatsyn 1 lambs, with a particular advantage in relative terms over the dry summer period (Table 3.2, Appendix 3.4). From 24/1/96 to 4/3/96 there was a 61% advantage to Aries HD. Carcass weight gain of lambs grazing Aries HD was 32% higher than that of lambs grazing Yatsyn 1 ryegrass (Table 3.2). This resulted in a 9% greater carcass weight at slaughter for Aries HD lambs over Yatsyn 1 lambs. Fat depth (GR score) was 24% higher in Aries HD lambs at equivalent carcass weight. The better performance of Aries HD lambs was not reflected in a significant difference in wool production.

Table 3.2 Cultivar effects on liveweight gain, stocking rate, carcass weight gain and composition, and wool production of lambs.

Measurement	Aries HD	Yatsyn 1	SEM	P
Lamb Growth Rate (g/day)				
11/12/95 to 24/1/96 (45 days) (8 lambs/plot)	130	111	8.7	0.2862
24/1/96 to 4/3/96 (40 days) (6.4 lambs/plot)	50	31	7.8	0.0351
4/3/96 to 25/4/96 (51 days) (6.4 vs 7.6 lambs/plot for Aries HD and Yatsyn 1 swards)	114	105	7.5	0.0922
11/12/95 to 25/4/96 (136 days)	104	84	4.6	0.1028
Mean Lambs/Plot				
(11/12/95 - 11/4/96)	6.9	7.4	0.4	0.5081
Carcass Weight ¹ (kg)	16.8	15.4	0.35	0.0056
Carcass Weight Gain (g/day)	45	34	2.67	0.0054
Dressing Out%	45.5	44.5	0.55	0.3486
Fat Depth ² (mm)	8.9	7.2	0.59	0.0383
Greasy Fleece Weight (kg)	1.95	1.88	0.075	0.3596
Clean Fleece Weight (kg)	1.63	1.57	0.067	0.4580

1 Adjusted to equal initial carcass weight by analysis of co-variance

2 Adjusted to equal carcass weight by analysis of co-variance

Chromium release rate from capsules was significantly greater in Aries HD lambs than in Yatsyn 1 lambs (Table 3.3). Herbage intake calculated using these release rates was 12% higher in Aries HD lambs, but this difference was not statistically significant, and was eliminated when intakes were re-calculated assuming the same release rate for both treatments. *In vitro* organic matter digestibility of herbage samples from oesophageal fistulates did not differ significantly between cultivars. Total daylight grazing time was about 8.8 hours for both cultivars, with lambs spending 1.8 hours ruminating (Table 3.3). Bite rate was on average 51 bites/minute. There was twice as much incidence of

clinical ryegrass staggers among Yatsyn 1 lambs as in those grazing Aries HD pasture (Table 3.3).

Table 3.3 Cultivar effects on herbage intake (19/4/96 to 25/4/96), chromium release rate, organic matter digestibility from oesophageal fistulates, grazing behaviour and the percentage of lambs with ryegrass staggers (24/1/96 to 4/3/96).

Measurement	Aries HD	Yatsyn 1	SEM	P
Herbage Intake (kg OM/day)	1.18	1.05	0.046	0.1624
Organic Matter Digestibility (%)	79.5	78.6	0.92	0.4113
Capsule Release Rate (mg/day)	165	155	11.5	0.0002
Grazing behaviour:				
Total grazing time (hours/12 hour day)	8.6	8.9	0.28	0.6175
Total ruminating time (hours/12 hour day)	1.6	1.8	0.16	0.2167
Rate of biting (bites/minute during day)	51.1	51.6	0.96	0.4687
Lambs With Severe Ryegrass Staggers (%)	15.0	29.0	NA	0.0271

In vitro organic matter digestibility, nitrogen and neutral detergent fibre content from herbage cuts or plucked samples did not differ significantly overall (Table 3.4), and showed no indications of seasonal differences between cultivars (Appendix 3.5). Mean peramine content in Yatsyn 1 was 26% greater than in Aries HD samples (Table 3.5). Lolitrem B content did not differ between cultivars, with peak levels detected on 21/2/96 at 1.63 vs 1.56 ppm for Aries HD and Yatsyn 1 samples respectively (Figure 3.2). Ergovaline contents in Aries HD herbage samples were consistently half those of Yatsyn 1 samples.

Table 3.4 Cultivar effects on herbage *in vitro* organic matter digestibility, nitrogen and neutral detergent fibre content.

Nutritive Value Estimates	Aries HD	Yatsyn 1	SEM	P
Herbage Cuts ¹				
Organic Matter Digestibility (%)	58.7	58.9	0.44	0.4821
Neutral Detergent Fibre (%)	59.9	60.1	0.96	0.8247
Nitrogen (%)	1.82	1.75	0.064	0.2190
Pluck Samples ²				
Organic Matter Digestibility (%)	66.3	67.1	2.1	0.6320
Neutral Detergent Fibre (%)	53.9	53.5	1.18	0.6499
Nitrogen (%)	2.65	2.74	0.111	0.3033

1 Mean from five cuts to ground level – 27/12/95 to 19/4/96

2 Mean from five bulked pluck samples - 27/12/95 to 19/4/96

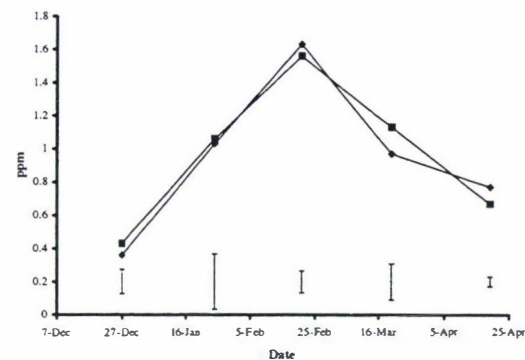
Table 3.5 Herbage endophyte alkaloid estimates (ppm).

Alkaloid	Aries HD	Yatsyn 1	SEM ¹	P
Lolitre B	0.95	0.97	0.197	0.8875
Peramine	11.85	15.92	0.992	0.0258
Ergovaline	0.23	0.42	0.042	0.0258

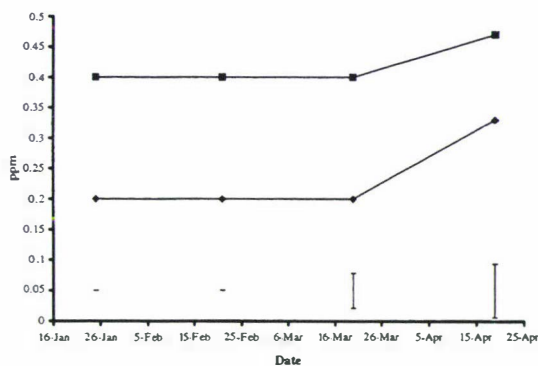
1 Mean from five cuts to ground level – 27/12/95 to 19/4/96

Figure 3.2 Alkaloid concentrations in herbage cut to ground level in Aries HD (◆) and Yatsyn 1 (■) pasture over the experiment: **A**, Lolitrem B; **B**, Ergovaline; **C**, Peramine.

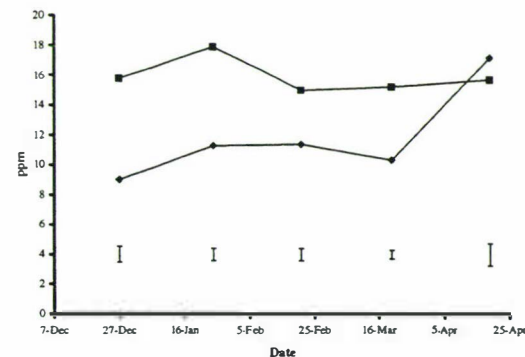
A



B



C



3.5 DISCUSSION

Few experiments have evaluated grass cultivars within a single species, in terms of animal production under continuous stocking. However, in an assessment of contrasting perennial ryegrasses under continuous sheep stocking, Davies *et al.* (1991, 1993) demonstrated large and significant differences between cultivars in terms of lamb production and related them to differences in organic matter digestibility.

Wrightson Seeds Ltd have demonstrated slightly higher levels of organic matter digestibility in Aries HD than in Yatsyn 1 pastures cut to grazing height every four weeks and allowed to regrow. However, under continuous sheep grazing management we were unable to repeat this effect. *In vitro* organic matter digestibility from herbage cuts, pluck samples and oesophageal fistulates, and estimates of herbage nitrogen and neutral detergent fibre were all similar between cultivars. These conflicting results may reflect a greater opportunity for stem development in intermittently defoliated pastures. The lack of difference under set stocking was not due to a dilution effect from weed species because perennial ryegrass content was high and similar for each cultivar. Similar estimates of herbage intake reflected similar nutritive value in the two cultivars.

The results suggest that seasonal contrasts in animal performance may have reflected differing alkaloid concentrations from the respective ryegrass/endophyte associations and effects on the incidence of ryegrass staggers. However, the liveweight gain advantage to Aries HD lambs was maintained when results for non-stagging lambs were compared (49 vs 24 ± 11.7 g/day for Aries HD and Yatsyn 1 lambs, respectively from 24/1/96 to 4/3/96). Liveweight gains have been reduced on endophyte infected ryegrass in the absence of clinical ryegrass staggers (Eerens *et al.* 1992). The ergovaline content detected in Aries HD herbage samples was consistently half that of Yatsyn 1 samples.

Lolitrems B has been regarded as the major alkaloid responsible for ryegrass staggers (Gallagher *et al.* 1984), but levels detected in the two cultivars were similar. Piper and

Fletcher (1990) concluded that ergovaline was not involved with the staggers syndrome. Ergovaline has been associated with reduced live weight gain in tall fescue (Belesky *et al.* 1988; Debessai *et al.* 1993) and perennial ryegrass (Piper *et al.* 1990; Fletcher *et al.* 1991; Fletcher and Sutherland 1993), elevated body temperature, respiration rate and reduced serum prolactin levels (Fletcher 1993). Also ryegrass/endophyte associations free of, or with low levels of lolitrem B but with significant levels of the tremogenic mycotoxin paxilline were found to be correlated with ryegrass staggers (Fletcher 1991; Fletcher and Sutherland 1993; Fletcher *et al.* 1993).

Levels of pasture production, estimated indirectly from the stocking rate required to maintain swards at 6 cm surface height, were similar for the two cultivars (Figure 3.1). This probably reflects the fact that peramine levels in both cultivars were greater than 10 ppm (Table 3.2 and Figure 3.2), the threshold thought to deter feeding by adult and larval Argentine stem weevil (Rowan *et al.* 1990). Endophyte infection levels were also high and consistent between cultivars.

3.6 CONCLUSIONS

Liveweight gain differences between perennial ryegrass cultivars can be due to quality factors, the association with endophyte, or both. Under continuous sheep grazing management the higher liveweight and carcass weight gains achieved on Aries HD pasture than on Yatsyn 1 pasture could not be attributed to any difference in herbage nutritive value. Better animal performance in this experiment reflected interrelated effects of alkaloid concentrations and ryegrass staggers. This highlights the need for animal evaluation experiments in assessing not only nutritive value, but also the effects of cultivar/endophyte associations which influence the production and balance of alkaloids.

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CHAPTER 4

EXPERIMENT 2: EVALUATION OF THE FEEDING VALUE OF ARIES HD PERENNIAL RYEGRASS (*Lolium perenne*).

1. PERFORMANCE OF LACTATING EWES IN SPRING

4.1 ABSTRACT

The feeding value of Aries HD perennial ryegrass (*Lolium perenne* L.) was evaluated over spring (3/9/96 to 1/12/96) using Romney ewes with suckling single lambs, with Yatsyn 1 perennial ryegrass as the control cultivar. There were three replicate plots of each cultivar of 0.33 ha continuously stocked with 6 ewes/plot after lambing to maintain a mean sward surface height of 6 cm. Ewes were removed from the area on 5 November at weaning. Ewe liveweight gain was significantly higher on Aries HD than Yatsyn 1 over September (94 vs 56 ± 14.5 g/day, $P < 0.062$) which coincided with a significantly higher herbage intake (1.78 vs 1.37 ± 0.038 kg DM/day, $P < 0.0322$). The percentage of ryegrass leaf was higher in Aries HD pasture, as was ewe bite rate, and calculated bite weight. The time spent grazing on these ryegrass cultivars was between 11.00 and 11.30 hours per 24 hour period. There was no significant difference in growth rates of suckling lambs between cultivars. Information on pasture nutritive value, herbage intake and grazing behaviour are discussed in relation to the differences in ewe performance.

This chapter forms the basis of a paper for submission to: *New Zealand Journal of Agricultural Science*.

4.2 INTRODUCTION

Herbage feeding value can be defined as the animal performance response to the total herbage consumed, or the worth of a herbage in terms of its animal production (Ulyatt 1981a and b; Laidlaw and Reed 1993). Thus animal production is the result of complex interactions between constituents of the herbage, the physiological stage of the animal, the gut microflora and the environment (Freer 1981). Differences in comparative feeding value can be assessed in grazing trials where intake is not limited by herbage availability (Ulyatt 1981a). The proportion of a pasture plant that is digested (apparent digestibility %) is a major component of nutritive value (Ulyatt 1981b). Digestibility and intake are considered to be the most serious quality limitations to animal production from pasture (Hacker 1982). Any genetic gain in digestibility must be validated in animal trials (Laidlaw and Reed 1993).

As grasses flower and mature there is a decline in digestibility because of an increased content of lignified cell walls and a decrease in the ratio of leaf to stem (Norton 1982). An improvement in digestibility by breeding may operate through a number of pathways including later flowering, greater leafiness, reduced lignification or higher proportion of cell contents (Hacker 1982; Vogel and Sleper 1994). Wrightson Seeds Ltd have specifically selected Aries HD perennial ryegrass for improved whole plant digestibility and have demonstrated an improvement in *in vitro* organic matter digestibility of 2.6% units over Yatsyn 1 perennial ryegrass in summer (Appendix 1.1).

In a lamb finishing experiment in the summer of 1995/96 (Chapter 3) it appeared that the better animal performance achieved on Aries HD pasture than on Yatsyn 1 pasture was due to the effects of endophyte alkaloid concentrations and associated ryegrass staggers, and could not be attributed to any differences in nutritive value. The aim of this experiment was to assess the feeding value of Aries HD perennial ryegrass using ewes under high physiological demand in spring, before the effect of endophyte alkaloids were apparent.

4.3 MATERIALS AND METHODS

4.3.1 Site

The experiment was conducted on the Sheep and Beef Cattle Research Unit, Massey University, Palmerston North. Clover-free swards of Aries HD and Yatsyn 1 were established in the autumn of 1995, in a randomised complete block design with three blocks (0.33 ha/plot). The experiment began on 3 September 1996 and concluded on 2 December 1996.

4.3.2 Animals

A total of 36 Romney ewes with suckling single lambs were selected in late August from 100 ewes which were synchronised during oestrus in March. Those diagnosed by ultrasound as carrying single lambs were managed as one mob prior to lambing. Lambing began on 22 August and was completed by 26 August. Lambs were tagged at birth and their birthweight recorded. Six ewes and their lambs were allocated to each plot on 3 September balanced for lamb sex and stratified according to lamb liveweight. Male lambs were castrated and all lambs were docked using rubber rings. Ewes were drenched monthly with Leviben (ricobendazole and levamisole hydrochloride; Youngs Animal Health NZ, Ltd) to control internal parasites. Lambs were drenched at 10 weeks of age. Sheep were given a footbath of zinc sulphate solution on 2 October to prevent footrot. Lambs were weaned on 5 November when ewes were removed from the experiment. A total of 6 lambs per cultivar were slaughtered on 6 December to provide a covariate of initial carcass weight for the summer lamb finishing experiment which followed (Chapter 5).

4.3.3 Pastures

Plots were grazed continuously to a sward surface height of 6 cm to ensure optimum herbage intake and to maintain equal sward conditions between plots (Hodgson 1990). This height was monitored twice per week with a sward stick (Barthram 1986), and maintained by adjusting ewe numbers between plots or with the addition of non-experimental dry sheep. There were monthly applications of urea or Cropmaster 15 (Ravensdown Fertiliser Co-operative Ltd, New Zealand), supplying a total of 109 kg N/ha, 15 kg P/ha, 15 kg K/ha and 12 kg S/ha over the period of the experiment. A general view of the swards is shown in Plate 4.1.

4.3.4 Pasture Measurements

Herbage mass was determined fortnightly by cutting six 0.1 m² quadrats per plot to ground level using an electric shearing hand-piece. The herbage samples were then washed, oven-dried at 80 °C for 24 hours and weighed individually. Botanical composition was determined fortnightly on bulked ground level samples from each plot. The samples were separated into ryegrass, other species and dead material. Leaf and stem percentage was determined monthly from ryegrass tillers. Herbage cuts to ground level and hand plucked samples to simulate herbage selected, were taken fortnightly for analysis of *in vitro* organic matter digestibility (Roughan and Holland 1977), nitrogen (Kjeldahl procedure) and neutral detergent fibre content (Van Soest 1994). Herbage cuts to ground level from October were analysed for concentrations of lolitrem B and ergovaline by HPLC (Baker et al. 1993). All herbage samples were frozen at -20°C and freeze dried before grinding through a 1 mm mesh screen. Tiller density was calculated from 40 pasture cores per plot, each 50 mm in diameter, taken at random, and the number of ryegrass and other species (predominantly *Poa annua*) tillers/core recorded (Mitchell and Glenday 1958).

Plate 4.1 General view of swards in Experiment 4.



Plate 4.2 Oesophageal fistulated sheep with bag fitted ready for sampling.



4.3.5 Animal Measurements

Ewe and lamb performance

Ewe and lamb liveweight was recorded fortnightly. Wool growth of ewes was measured by clipping mid-side samples (10cm x 10cm area) to skin level at the beginning and end of the experiment (Bigham 1974). The wool yield of the patches was determined (clean weight/greasy weight) as described by Min et al (1998). Lamb wool growth was measured by shearing the lambs at the end of the experiment.

Herbage Intake

Herbage intake was measured during peak lactation from 23 September to 4 October, using intra-ruminal slow release chromium sesquioxide capsules (Captech New Zealand Ltd, Auckland) as described by Parker et al. (1989). Chromium capsules were orally administered to each ewe on 16 September. Faecal grab samples were collected daily from each ewe, over the period 9-17 days after capsule administration. They were oven dried at 80 °C, bulked across days for each animal on an equal dry weight basis and ground. Two sheep fistulated in the oesophagus (Plate 4.1) were rotated among the swards, to collect samples of the herbage selected (six samples/cultivar) for estimation of *in vitro* organic matter digestibility (Roughan and Holland 1977). The extrusa samples were immediately placed in an ice box, frozen and stored at -20°C. They were then freeze dried, and ground to pass a 1 mm mesh screen before laboratory analysis. Chromium release rate was estimated from chromium capsules recovered from lambs at slaughter in the previous experiment (Chapter 3).

Grazing time, bite rate and bite weight

Measurements of grazing behaviour were made during the herbage intake measurement period. Two 24-hour profiles were completed, each spread over four six-hour periods (0600-1200, 1200-1800, 1800-2400, 2400-0600 h) during each week, coinciding with the faecal sampling periods. Observations were recorded every 10 minutes for the

number of ewes grazing, ruminating or idling in each plot (Jamieson and Hodgson 1979). During darkness an infrared nightscope was used to aid in the identification grazing activity. Total time spent grazing was calculated by adding together observations for the four periods. Bite rates (time taken for 20 bites) were recorded on random animals in each plot, between each grazing observation during daylight hours (Jamieson and Hodgson 1979). Bite weight (g OM/bite) was calculated using measured values for herbage intake (g OM/day), grazing time (min/24 hours) and bite rate (bites/min) as described by Kusmartono et al. (1996).

4.3.6 Statistical analysis

Analysis of variance and repeated measures analysis were carried out using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc. 1988). All statistical analyses were based on plot mean values with three replicates. Individual animal performance was calculated using data from core group animals grazing each plot and least squares means analysis used where numbers were unbalanced. Factorial analysis of variance was used to analyse herbage intake and proportion of time spent grazing, ruminating and resting, with the factors being cultivar, week and their interaction. Factorial analysis of variance was used to analyse *in vitro* organic matter digestibility samples from oesophageal fistulated sheep, with the factors being cultivar, sheep and their interaction.

4.4 RESULTS

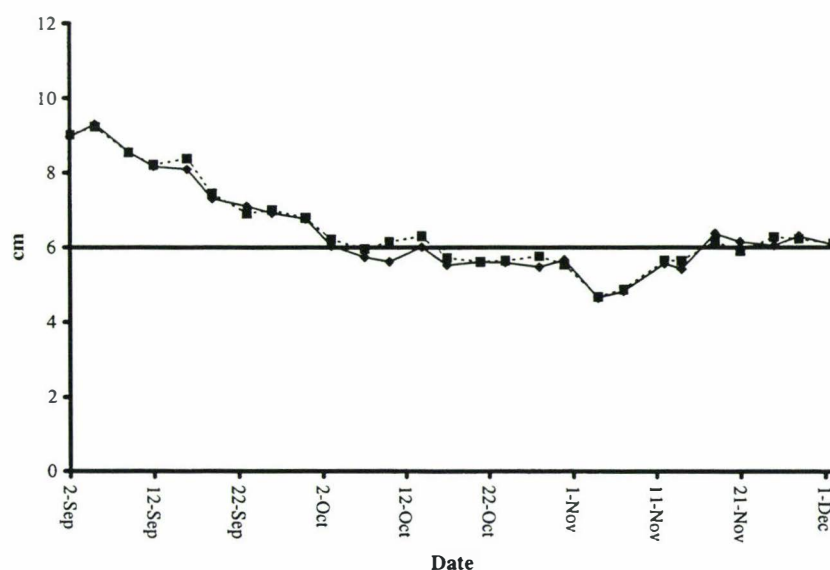
Weather conditions over the experiment are given in Appendix 4.1. Mean herbage mass and sward height was similar over the spring for the two cultivars (Table 4.1). Swards were maintained at a 6-cm mean sward height (Figure 4.1). For both cultivars the ryegrass component of the pasture was about 55% in live herbage, with the major weed species being *Poa annua*, measured in botanical composition and tiller density comparisons. The percentage of ryegrass leaf on average was slightly higher in Aries HD than in Yatsyn 1 pastures. *In vitro* organic matter digestibility and neutral detergent fibre content from herbage on offer (ground level herbage cuts) and herbage selected

(plucked herbage samples) did not differ significantly between cultivars (Table 4.2, Appendix 4.2). There was a small difference in nitrogen content which approached significance in herbage cut samples, but not in plucked samples (Table 4.2). Lolitrem B levels were low in both cultivars and ergovaline was barely detectable (Table 4.3).

Table 4.1 Herbage mass, sward height, botanical composition (mean from entire experiment), tiller density (17/10/96), for two cultivars of perennial ryegrass grazed by ewes in spring.

Measurement	n/trt	Aries HD	Yatsyn 1	SEM	P
Herbage mass (kg DM/ha)	21	2890	3020	246	0.4205
Sward height (cm)	81	6.4	6.3	1.90	0.8653
% Ryegrass in live herbage	21	57	54	5.0	0.3885
% Leaf in ryegrass	12	63	61	0.7	0.0352
Ryegrass tiller density (tillers/m ²)	3	3160	2730	276	0.3876
Poa tiller density (tillers/m ²)	3	8900	7940	930	0.5418

Figure 4.1 Mean sward height for Aries HD (◆) and Yatsyn 1 (■) pasture and 6-cm target line.



The *in vitro* organic matter digestibility of herbage sampled from oesophageal fistulated sheep did not differ significantly between cultivars (Table 4.4). Ewe herbage intake on Aries HD pasture was greater at 1.72 kg DM/day than Yatsyn 1 at 1.37 kg DM/day ($P < 0.05$) using separate release rates from the previous experiment (165 vs 155 ± 3.1 mg/day, $P < 0.0005$ for Aries HD and Yatsyn 1 pasture respectively). The total proportion of time spent grazing in a 24-hour period was between 11 and 11.3 hours on both ryegrass cultivars. Mean bite rate per minute was significantly higher on Aries HD than on Yatsyn 1 pasture ($P = 0.08$).

Table 4.2 Organic matter digestibility, neutral detergent fibre and nitrogen content in herbage samples over the entire experiment.

	Aries HD	Yatsyn 1	SEM	P
Herbage cut samples ¹	(n=12)	(n=12)		
Organic matter digestibility (%)	68.3	67.2	1.83	0.4764
Neutral detergent fibre (% DM)	53.6	55.5	1.49	0.2185
Nitrogen (% DM)	2.28	2.09	0.078	0.0773
Herbage pluck samples ²	(n=9)	(n=9)		
Organic matter digestibility (%)	73.9	74.0	1.65	0.9184
Neutral detergent fibre (% DM)	43.6	43.2	1.73	0.7373
Nitrogen (% DM)	3.71	3.67	0.100	0.6606

1 Mean from four sample dates - 6/9/96 to 28/11/96

2 Mean from three bulked pluck samples - 3/9/96 to 10/11/96

Table 4.3 Cultivar effects on alkaloid concentrations (ppm) in October 1996.

Alkaloid	Aries HD	Yatsyn 1	SEM	P
Number of samples	3	3	-	-
Lolitrein B	0.23	0.10	0.024	0.0572
Ergovaline	0.00	0.07	0.024	0.1835

Over the spring the ewes with suckling single lambs gained weight with a 30% advantage to Aries HD (Table 4.5). Over September Aries HD ewes gained almost 40 g/day more than Yatsyn 1 ewes ($P=0.06$). This enabled Aries HD ewes to gain an extra kilogram over the spring months (4.36 kg versus 3.33 kg total liveweight gain for Aries HD and Yatsyn 1 respectively). Wool growth in both the ewes and the lambs was similar between cultivars. The liveweight gain of the suckling lambs did not differ between cultivars at any stage (Table 4.6), but decreased as the experiment progressed. Carcass weights lambs from the two cultivars were similar (Table 4.6). Liveweight over time for ewes and lambs is shown in Appendix 4.3 and 4.4. Stocking rate was similar between cultivars at all stages of the experiment.

Table 4.4 Cultivar effects on *in vitro* organic matter digestibility measured using oesophageal fistulates, herbage intake and grazing behaviour of ewes from 23 September to 4 October 1996.

	Aries HD	Yatsyn 1	SEM	P
No. of ewes	17	18	-	-
Herbage intake (kg OM/day)	1.72	1.37	0.038	0.0322
Organic matter digestibility (%)	79.8	78.1	1.43	0.3600
Grazing behaviour:				
Total grazing time (hours/24 hours)	11.3	11.1	0.17	0.1012
Daily ruminating time (hours/day)	2.5	2.3	0.05	0.3773
Rate of biting (bites/minute during day)	43.0	41.7	0.53	0.0802

Table 4.5 Perennial ryegrass cultivar effects on ewe liveweight gain and wool production over spring.

Ewe performance	Aries HD	Yatsyn 1	SEM	P
No. of ewes	18	18	-	-
Liveweight gain (g/day)				
3/9/96 to 2/10/96 (29 days)	94	56	14.5	0.0624
2/10/96 to 5/11/96 (34 days)	48	50	11.2	0.5674
3/9/96 to 5/11/96 (63 days)	69	53	9.3	0.1203
Mean ewes/plot	6.4	6.7	0.66	0.8031
Wool growth (mg/0.01m ² /day)	96	97	10.4	0.9641

Table 4.6 Perennial ryegrass cultivar effects on lamb liveweight gain and wool production over spring.

Lamb performance	Aries HD	Yatsyn 1	SEM	P
No. of lambs	18	18	-	-
Liveweight gain (g/day)				
3/9/96 to 2/10/96 (29 days)	278	277	10.7	0.9045
2/10/96 to 5/11/96 (34 days)	213	205	7.9	0.7073
5/11/96 to 1/12/96 (26 days)	125	134	8.0	0.6408
3/9/96 to 1/12/96 (89 days)	203	203	5.5	1.0000
Mean lambs/plot	6.0	6.0	-	-
Total greasy wool (kg)	1.35	1.37	0.05	0.8823
Carcass weight (kg) n= 6/trt	11.3	11.4	0.51	0.9226

4.5 DISCUSSION

The experiment was designed to determine the feeding value of Aries HD perennial ryegrass using Yatsyn 1 as the control cultivar, with ewes under a high physiological demand for nutrients. The factors contributing to herbage feeding value are nutritive value (as measured here by *in vitro* digestibility) and voluntary feed intake. Therefore animal production can be increased by either increasing the intake (quantity eaten) or by increasing the nutritive value (quality eaten) of the herbage.

Cultivars were sown alone for the comparison of animal performance. However, *Poa annua* was a major (but similar) weed contaminant in both cultivars. Bircham and Hodgson (1982) found that in their continuously grazed swards, *Poa annua* tended to be grazing-evasive, retaining leaf area and mass because its leaves were less accessible to defoliation than those of its competitors. Therefore *Poa annua* was probably not a major contributor to the diet, but may have diluted contrasts between cultivars.

Herbage intake of the ewes was measured during peak lactation (23 September to 4 October) two to four weeks after lambing when the demand for nutrients was the greatest. Ewe intake estimates showed a relatively higher intake on Aries HD than on Yatsyn 1 pasture (Table 4.4). In actual terms, estimates were low compared to predictions made based on energy requirements (2.4 kg OM/day; Geenty and Rattray 1987). It is possible that *in vitro* organic matter digestibility was underestimated, since at high levels of digestibility small errors in estimates can exert a disproportionate influence on calculation of herbage intake (Parker et al. 1990). If small differences in digestibility are to be measured, careful monitoring of the diet selected between groups is required because of individual animal variation in diet selection or digestive efficiency (Parker et al. 1990).

The proportion of time spent grazing for both cultivars was greatest between midday and 6:00 pm. Time spent grazing at night was minimal. Bite rate was significantly higher on Aries HD than on Yatsyn 1 pasture, perhaps reflecting the higher proportion of ryegrass leaf in this spring pasture (Table 4.4). The digestibility of ryegrass stem

declines at a much faster rate than that of the leaf (Terry and Tilley 1964). However, the slightly greater leaf content of Aries ryegrass in this study was not reflected in higher digestibility of either whole sward, plucked samples or oesophageal fistulated sheep samples. Calculated ewe bite weight was 59 vs 49 mg for Aries HD and Yatsyn 1, respectively.

Suckling lamb growth rates were high and similar between cultivars, presumably reflecting high and non-limiting milk yields on both cultivars. Ewe performance was significantly better on Aries HD pasture reflecting the higher herbage intake on this cultivar. In previous work in Chapter 3 greater lamb growth rates over summer and autumn in Aries HD than Yatsyn 1 were attributed to lower endophyte (*Neotyphodium lolii*) alkaloid production from Aries HD pasture rather than differences in herbage intake. However, differences in ewe liveweight gain in this experiment would not have reflected endophyte alkaloids because of the low concentrations found in spring pasture samples. Lolitrem B levels in herbage cut to ground level were < 0.30 ppm and only trace amounts of ergovaline were detected (Table 4.3). Cases of ryegrass staggers from June to October have not been reported in the literature, when lolitrem B concentrations are usually < 1 ppm (di Menna et al. 1992; Blythe 1993). Eerens et al. (1997) has reported significantly higher lamb liveweight gains between birth and weaning on endophyte-infected than on endophyte-free perennial ryegrass, recorded in spring when levels of alkaloids are relatively low.

4.6 CONCLUSIONS

Ewes with suckling lambs had a higher bite rate and herbage intake on Aries HD than on Yatsyn 1 pasture. Percentage of ryegrass leaf was higher in Aries HD swards. The feeding value of Aries HD perennial ryegrass was superior to that of Yatsyn 1 under continuous grazing management over spring as determined by better liveweight gain of ewes.

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CHAPTER 5

EXPERIMENT 3: EVALUATION OF THE FEEDING VALUE OF ARIES HD PERENNIAL RYEGRASS (*Lolium perenne*). 2. PERFORMANCE OF WEANED LAMBS IN SUMMER AND AUTUMN

5.1 ABSTRACT

The feeding value of Aries HD perennial ryegrass (*Lolium perenne*) was evaluated over summer (2/12/96 to 12/3/97) using Romney weaned lambs, with Yatsyn 1 perennial ryegrass as the control cultivar. There were three replicate plots of each cultivar of 0.33 ha continuously stocked with 8 lambs/plot to maintain a mean sward surface height of 6 cm. There was no significant difference in lamb liveweight gain (116 vs 111 ± 5.1 g/day, $P = 0.6542$) or herbage intake (0.7 vs 0.6 ± 0.10 kg OM/day, $P = 0.5715$) between Aries HD and Yatsyn 1 lambs respectively. Liveweight gain decreased as the experiment progressed from 174g/day in December, 102 g/day in January/February to 76 g/day in February/March. Incidence of ryegrass staggers was low with 9% of Yatsyn 1 lambs being affected over late February/early March, which coincided with a peak lolitrem concentration of 1.37 ppm in Yatsyn 1 pasture. There was no significant difference in final carcass weights of lambs (14.9 vs 14.6 ± 0.24 kg, $P = 0.5807$) reflecting the similar nutritive value and alkaloid concentrations between the two cultivars over this summer/autumn period.

This chapter forms the basis of a paper for submission to: *New Zealand Journal of Agricultural Science*.

5.2 INTRODUCTION

The feeding value of a pasture can be improved by increasing the proportion of cultivars of high nutritive value and through management that promotes vegetative growth (Ulyatt 1981). A line of perennial ryegrass selected for low leaf shear strength was digested more rapidly in the rumen, but under field conditions there was no significant difference in intake or liveweight gain of sheep (Inoue et al. 1993). Cultivars with a higher water-soluble carbohydrate concentration have increased organic matter digestibility and feeding value (Frame 1992). Late flowering cultivars of perennial ryegrass when compared at the same calendar date have a higher digestibility and leaf to stem ratio than early flowering cultivars because of their slower rate of development (Vogel and Sleper 1994). However, when they are compared at flowering early varieties are usually more digestible (Hacker 1982). Increased lamb production has been demonstrated on tetraploid ryegrass cultivars due to their higher organic matter digestibility than diploid cultivars at the same stage of maturity (Davies et al. 1993; Ryan and Widdup 1997). Aries HD was selected for increased organic matter digestibility over the summer when the nutritive value of perennial ryegrass is typically low. Organic matter digestibility is the only criterion that integrates all the components of digestibility, therefore if genetic variation exists for any one of these components then the forage is likely to be improved (Vogel and Sleper 1994).

All comparisons of perennial ryegrass cultivars in New Zealand must be interpreted with respect to their endophyte levels. The perception of genetic differences between cultivars in field performance has been impaired by the presence of the endophyte (Easton 1983; Fletcher and Easton 1997). Every cultivar/endophyte association results in a unique combination of alkaloids and animal responses (Powell et al. 1993). The presence of endophyte can lead to misinterpretation of plant evaluation data because it increases biotic and abiotic host plant tolerance and causes toxicosis in grazing animals due to alkaloids (Ravel et al. 1997). Endophyte effects on animals include reduced liveweight gain, ryegrass staggers, increased faecal moisture and soiling, increased incidence of flystrike, increased rectal temperatures and respiration rates, and depressed serum prolactin levels (Fletcher 1993a; Familton et al. 1995).

The first weaned lamb experiment over summer and autumn (Chapter 3) showed that contrasts in lamb performance between Aries HD and Yatsyn 1 reflected the interrelated effects of alkaloid concentrations and ryegrass staggers. Better lamb performance on Aries HD pasture could not be attributed to any difference in herbage nutritive value. Chapter 4 reported the performance of ewes with their lambs in spring, when the risks of endophyte alkaloids were low. Ewe liveweight gain was significantly higher on Aries HD than Yatsyn 1 over September (94 vs 56 g/day, $P < 0.062$) which coincided with a higher herbage intake and bite rate. There was also a significantly higher proportion of ryegrass leaf in Aries HD pasture. This paper describes the performance of weaned lambs over the following summer-autumn period. Herbage intake, grazing behaviour, nutritive value parameters, endophyte alkaloid concentrations and incidence of staggers are presented.

5.3 MATERIALS AND METHODS

5.3.1 Site

The experiment was conducted on the Sheep and Beef Cattle Research Unit, Massey University, Palmerston North. Clover-free swards of Aries HD and Yatsyn 1 were established in the autumn of 1995, in a randomised complete block design with three blocks (0.33 ha/plot). The experiment began 2 December 1996 and was concluded on 21 March 1997.

5.3.2 Animals

A total of 60 Romney weaned lambs were selected for the summer experiment in late November 1996 (30 ewe lambs and 30 wether lambs). This total consisted of the 36 lambs from the previous spring experiment (Chapter 4) plus 24 similar lambs selected from the Research Unit flock. Ten lambs were allocated to an initial slaughter group and their carcass weights were used to predict initial carcass weight of the remaining 48 lambs at the start of the experiment. The lambs from the previous spring experiment remained on their original replicate plots and the additional 24 lambs were allocated to a

stratified manner according to liveweight. Lambs were drenched monthly with Leviben (ricobendazole and levamisole hydrochloride; Young's Animal Health NZ Ltd) to control internal parasites, and dipped with Zenith (diflubenzuron; Young's Animal Health NZ, Ltd) in January to prevent fly strike.

5.3.3 Pastures

Plots were grazed continuously to a sward surface height of 6cm to ensure optimum herbage intake and to equal sward conditions between replicates (Hodgson 1990). This height was monitored twice per week with a sward stick (Barthram 1986), and was maintained by adjusting lamb numbers. There were monthly applications of urea or Cropmaster 15 (Ravensdown Fertiliser Co-operative Ltd, New Zealand), equivalent to a total of 109 kg N/ha, 15 kg P/ha, 15 kg K/ha and 12 kg S/ha over the period of the experiment. Pastures were irrigated with a sprinkler irrigation system from 17 December to 22 December, 6 January to 11 January and 3 February to 8 February 1997. A general view of the swards and an experimental lamb is shown in Plate 5.1 and 5.2.

5.3.4 Pasture Measurements

Measurements of herbage mass, botanical composition, tiller density and quality (*in vitro* organic matter digestibility, nitrogen and neutral detergent fibre) were made using identical methods to those employed during the preceding lactation experiment in this series (Chapter 4). Monthly herbage samples were also analysed for concentrations of lolitrem B, peramine and ergovaline by HPLC (Barker et al. 1993). Endophyte infection percentage was determined using ten tillers/plot on 27/3/97 by examining stained epidermal strips from leaf sheaths under a light microscope (Latch and Christensen 1985). Pasture was also cut monthly to 4 cm in a 2 m x 0.5 m strip from under one exclusion cage permanently sited in each plot. Pasture accumulation rate was calculated as the difference between final and initial herbage mass divided by the regrowth period. Tiller density was calculated from 40 pasture cores per plot, each 50 mm in diameter, taken at random, and the number of ryegrass and other species (predominantly *Poa annua*) tillers/core recorded (Mitchell and Glenday 1958).

5.3.5 Animal Measurements

Lamb performance

Lamb liveweight was recorded fortnightly. All lambs were slaughtered on 25 March 1997 to record carcass weight, dressing out percentage, and fat depth (GR: Kirton 1989). Initial carcass weight of the experimental lambs was predicted from regression based on 12 lambs slaughtered at the beginning of the experiment (Chapter 4). Carcass weight gain was estimated using the initial estimated carcass weight and the final carcass weight. Dressing out percentage was calculated by using the final carcass weight and fasted weight of lambs before slaughter.

Lambs were shorn at the beginning of the experiment and one week prior to slaughter. Greasy wool was weighed and a grab sample collected from each fleece, conditioned at 20 °C and 65% relative humidity for 48 hours, scoured and weighed. The wool yield (clean weight/greasy weight) was then determined as described by Min et al (1998), and clean wool growth calculated.

Animal health

Lambs were scored for severity of ryegrass staggers on a 0 – 5 scale (Appendix 2.1). Staggers was assessed fortnightly during weighing. Lambs unable to walk to weighing scales were transported. Rectal temperature was recorded on 19/2/97, 26/2/97 and 5/3/97 using digital clinical thermometers. Blood samples were drawn on 3/2/97 and 7/3/97 by jugular venipuncture into EDTA vacutainers and assayed for serum prolactin using the radioimmunoassay technique (Kirkwood et al. 1984). Rectal temperatures and blood samples were taken from sheep while they were in holding yards in the field.

Plate 5.1 General view of swards in Experiment 3 with sprinkler irrigation in the background.



Plate 5.2 A lamb in Experiment 3.



Intake and grazing behaviour

Measurements of herbage intake and grazing behaviour were made in two successive four-day periods in both January and March 1997. Herbage intake was estimated using identical methods to those reported in Chapter 4. The release rate of chromium was estimated by recovering chromium capsules at slaughter. Four sheep fistulated in the oesophagus were rotated among the swards, to collect samples of the herbage selected (12 samples/cultivar) for estimation of *in vitro* organic matter digestibility (Roughan and Holland 1977).

Observations of 24-hour grazing behaviour were made from a van parked in the plots. Grazing activities were recorded at intervals of 10 minutes during one continuous period of 24 hours in each four-day intake period. Observations were recorded for the number of ewes grazing, ruminating or idling in each plot (Jamieson and Hodgson 1979). During darkness an infrared nightscope was used to aid in the identification of grazing activities. Bite rates (time taken for 20 bites) were recorded on random animals in each plot during periods of peak grazing activity mid morning and mid afternoon (Jamieson and Hodgson 1979).

5.3.6 Statistical analysis

Analysis of variance and repeated measures analysis were carried out using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc. 1988). All statistical analyses were based on plot mean values with three replicates. Individual animal performance was calculated using data from core group animals and least squares means analysis used where numbers were unbalanced. Factorial analysis of variance was used to analyse herbage intake and proportion of time spent grazing, ruminating and resting, with the factors being cultivar, week and their interaction. The number of lambs with severe staggers was analysed using a chi-square test adjusted using a Yates correction factor (Little and Hills 1978).

5.4 RESULTS

The monthly mean rainfall and monthly maximum air temperature were similar to the 20-year average at the experimental site (Table 5.1, Appendix 5.1). A mean sward surface height of 6-cm was achieved for both cultivars (Figure 5.1). Mean herbage mass was similar between Aries HD and Yatsyn 1 pastures (4000 vs 4170 kg DM/ha). For both cultivars the ryegrass component of the pasture was about 57% in live herbage, with the major weed species being *Poa annua*, measured in botanical composition and tiller density comparisons (Table 5.2). The percentage of leaf in ryegrass was high (70%) and similar for both cultivars. Pasture accumulation rate under exclusion cages from December to March was on average 34 kg DM/ha/day for both cultivars. Endophyte infection of tillers was high in both cultivars (> 80%).

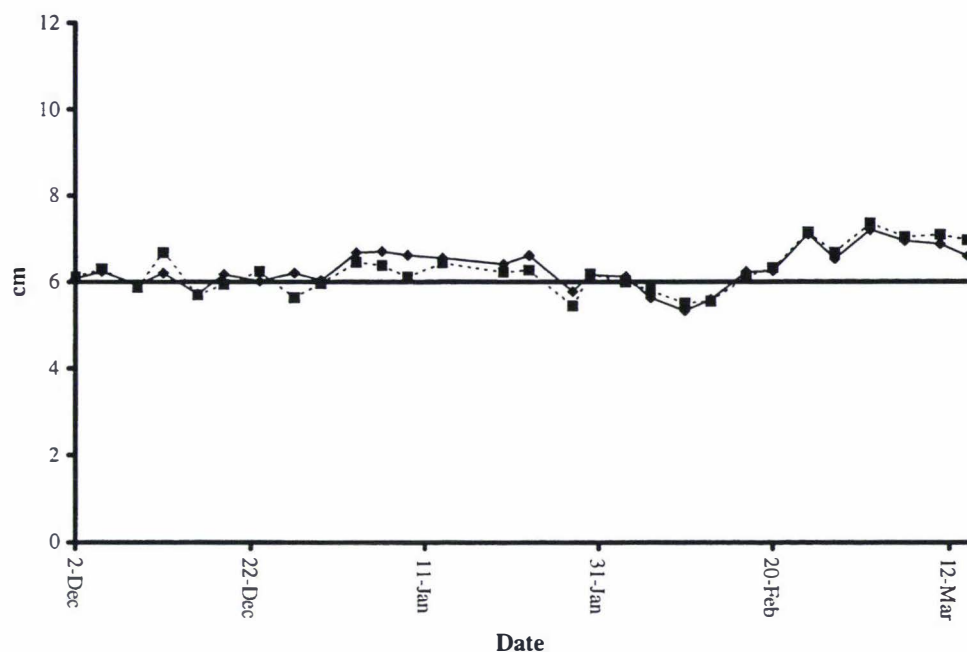
Table 5.1 Weather conditions at the experimental site during summer 1996/97.

Month	Monthly mean rainfall		Monthly mean maximum air	
	(mm)		temperature (°C)	
	1996/97	20-year mean	1996/97	20-year mean
December	91.1	84.4	20.2	20.5
January	68.0	63.5	21.0	22.3
February	58.0	70.0	22.0	22.7
March	68.1	80.0	19.9	20.9

Table 5.2 Herbage mass, sward height, botanical composition (mean from entire experiment), pasture accumulation rate (PGR) under cages (December to March), tiller density (15 January and 21 March) and the proportion of tillers infected with endophyte.

Measurement	n/trt	Aries HD	Yatsyn I	SEM	P
Herbage mass (kg DM/ha)	24	4000	4170	373	0.4558
Sward height (cm)	90	6.3	6.3	0.75	0.9028
% Ryegrass in live herbage	12	57	58	6.6	0.9264
% Leaf in ryegrass	12	73	72	4.2	0.8060
PGR under cages (kg DM/ha/day)	12	35	34	4.3	0.8316
Ryegrass tiller density (tillers/m ²)	6	4050	4110	760	0.9512
Poa tiller density (tillers/m ²)	6	11,390	11,420	328	0.9502
% Endophyte infection on 27/3/97	3	86.7	80.0	2.36	0.1835

Figure 5.1 Mean sward height for Aries HD (◆) and Yatsyn 1 (■) pasture and 6-cm target line.



In vitro organic matter digestibility, neutral detergent fibre and nitrogen content from herbage cut to ground level, pluck samples and cage cut samples did not differ significantly between cultivars (Table 5.3, Appendix 5.2 and 5.3). Lolitrem B concentration in herbage samples cut to ground level was similar in both cultivars (Table 5.4). The peak lolitrem B concentration detected (Figure 5.2) was on 24 February (1.16 vs 1.37 ppm for Aries HD and Yatsyn 1 respectively, $P = 0.5286$). Only trace amounts of ergovaline were detected in Yatsyn 1 herbage samples. Peramine levels were similar in both cultivars (14.23 vs 9.98).

Table 5.3 Organic matter digestibility, neutral detergent fibre and nitrogen content in herbage samples over the entire experiment.

	Aries HD	Yatsyn 1	SEM	P
	(n=15)	(n=15)		
Herbage cut samples ¹				
Organic matter digestibility (%)	61.4	61.8	2.15	0.7891
Neutral detergent fibre (% DM)	55.0	55.4	1.82	0.7849
Nitrogen (% DM)	2.14	2.08	0.068	0.2938
Herbage pluck samples ²	(n=12)	(n=12)		
Organic matter digestibility (%)	68.5	68.7	1.44	0.8449
Neutral detergent fibre (% DM)	48.8	48.3	0.53	0.3390
Nitrogen (% DM)	3.00	2.98	0.106	0.8268
Herbage cage Samples ³	(n=18)	(n=18)		
Organic matter digestibility (%)	77.6	76.9	2.26	0.6346
Neutral detergent fibre (% DM)	44.3	45.6	0.80	0.1045
Nitrogen (% DM)	3.06	2.72	0.234	0.1279

1 Mean from five sample dates - 28/11/96 to 27/3/97

2 Mean from four bulked pluck samples - 2/12/96 to 28/2/97

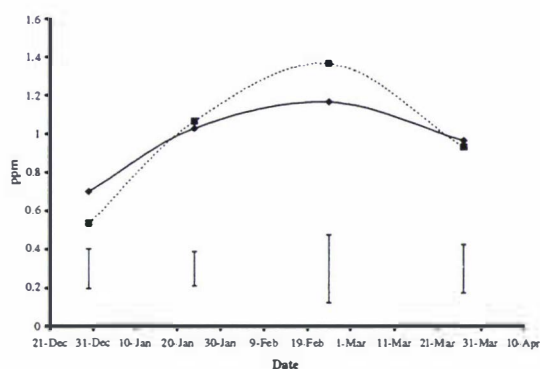
3 Mean from five sample dates - 14/11/96 to 2/4/97

Table 5.4 Herbage alkaloid estimates (ppm), mean from four sample dates (December to March).

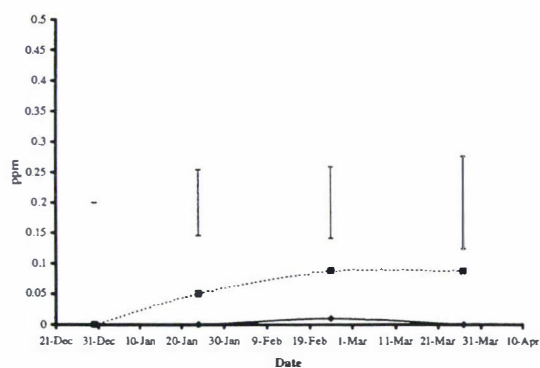
Alkaloid	Aries HD	Yatsyn 1	SEM	P
	(n=12)	(n=12)		
Lolitre B	0.97	0.98	0.159	0.9475
Peramine	9.98	14.23	3.608	0.2377
Ergovaline	0.00	0.06	0.024	0.0848

Figure 5.2 Alkaloid concentrations in herbage cut to ground level in Aries HD (◆) and Yatsyn 1 (■) pasture over the experiment: **A**, Lolitrem B; **B**, Ergovaline; **C**, Peramine.

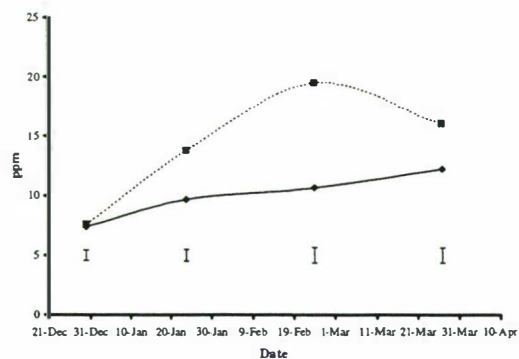
A



B



C



Herbage intake (Table 5.5) measured in January and March did not differ between Aries HD and Yatsyn 1 cultivars (0.65 vs 0.57 ± 0.095 kg OM/day, $P = 0.5715$). A mean chromium release rate of 141 mg/day was used to calculate intake (Table 5.5). Total grazing time was about 11 hours for both cultivars, with lambs spending just over 2 hours ruminating in the daytime. Bite rate was on average 48 bites/minute during daytime grazing. In March organic matter digestibility from oesophageal fistulate samples was significantly higher in Aries HD than in Yatsyn 1 pastures (73.6 vs $65.6 \pm 0.18\%$, $P = 0.08$), as was total grazing time (11.6 vs 11.2 ± 0.17 hours, $P = 0.01$). However, this difference was not reflected in any difference in herbage intake.

Table 5.5 Cultivar effects on herbage intake and grazing behaviour of lambs from 20 to 31 January and 11 to 21 March 1997.

Measurement	Aries HD	Yatsyn 1	SEM	P
No. of lambs	24	24	-	-
Chromium release rate (mg Cr/day)	139	142	2.6	0.4754
January				
Herbage intake (kg OM/day)	0.53	0.54	0.023	0.6252
Organic matter digestibility (%)	67.2	66.7	0.87	0.7817
Grazing behaviour:				
Total grazing time (hours/24 hours)	11.1	10.7	0.24	0.3454
Daily ruminating time (hours/day)	2.6	2.8	0.11	0.2563
Rate of biting (bites/minute during day)	49.5	50.5	0.67	0.1592
March				
Herbage intake (kg OM/day)	0.77	0.59	0.041	0.1824
Organic matter digestibility (%)	73.6	65.6	1.27	0.0818
Grazing behaviour:				
Total grazing time (hours/24 hours)	11.6	11.2	0.17	0.0109
Daily ruminating time (hours/day)	2.2	2.4	0.09	0.1631
Rate of biting (bites/minute during day)	46.0	46.9	1.40	0.3360

From December through to March lambs gained 114 g/day on both cultivars (Table 5.6). There was no difference in mean lamb numbers between cultivars. Lamb growth rate decreased as the experiment progressed from 174 g/day in December, 102 g/day in January/February to 76 g/day in February/March. Liveweight of the lambs over time is shown in Appendix 5.4. Carcass weight gain was about 40 g/day for both cultivars, with slaughtered lambs having a final carcass weight of just under 15 kg and a dressing out percentage was 45%. Fat depth (GR) was greater in Aries HD than in Yatsyn 1 lambs (8.4 vs 7.1 ± 0.33 mm, $P < 0.05$). Final clean fleece weight was about 1.5 kg for both Aries HD and Yatsyn 1 lambs.

Table 5.6 Cultivar effects on lamb liveweight gain, carcass weight gain and composition, and wool production over summer.

Ewe performance	Aries HD	Yatsyn 1	SEM	P
No. of lambs	24	24	-	-
Liveweight gain (g/day)				
3/12/96 to 30/12/96 (27 days)	171	177	7.2	0.6101
30/12/96 to 11/2/97 (43 days)	114	89	9.3	0.1399
11/2/97 to 12/3/97 (29 days)	67	84	10.8	0.2781
3/12/96 to 12/3/97 (99 days)	116	111	5.1	0.6542
Mean lambs/plot	8.3	8.1	0.31	0.7892
Carcass weight ¹ (kg)	14.9	14.6	0.24	0.5807
Carcass weight gain (g/day)	40	38	2.2	0.6924
Dressing out%	45.2	45.1	0.41	0.9112
Fat depth ² (mm)	8.4	7.1	0.33	0.0190
Greasy fleece weight (kg)	1.7	1.7	0.18	0.6802
Clean fleece weight (kg)	1.5	1.4	0.15	0.5535

1 Adjusted to equal initial carcass weight by analysis of co-variance

2 Adjusted to equal carcass weight by analysis of co-variance

The incidence of staggers (Table 5.7) was low in this experiment with just 9.4% of Yatsyn 1 lambs being carried to weighing in late February to early March ($P = 0.1087$). Only three lambs grazing Yatsyn 1 exhibited clinical staggers with a mean severity score of 3.0 over this period. Mean rectal temperature was 40.15 °C in Aries HD and Yatsyn 1 lambs recorded in late February and mid-March (Table 5.7). Mean air temperature was 17.4 °C with a maximum of 21.4 °C on measurement days. Serum prolactin concentration in early March was 69.5 ng/ml recorded on days with a mean air temperature of 17.7 °C and a maximum of 20.2 °C.

Table 5.7 Cultivar effects on staggers incidence (19 February to 12 March), rectal temperature (19 February to 12 March), and serum prolactin concentration (3 March and 7 March).

	n/trt mean	Aries HD	Yatsyn 1	SEM	P
Incidence of staggers					
(% of lambs with staggers)	4	0	9.4	na	0.1087
Rectal temperature (°C)	12	40.1	40.2	0.27	0.4965
Serum prolactin conc. (ng/ml)	6	73.1	65.8	38.76	0.6306

5.5

DISCUSSION

In Chapter 4 it was reported that the feeding value of Aries HD perennial ryegrass was superior to that of Yatsyn 1 as determined by significantly higher ewe liveweight gain which coincided with a higher herbage intake and bite rate. The objective of this experiment was to assess the feeding value of the same pastures over the proceeding summer-autumn period with weaned lambs.

In vitro organic matter digestibility did not differ between cultivars from both herbage cuts and hand-plucked samples, which supports observations in Experiment 1 (Chapter 3). Organic matter digestibility from oesophageal fistulate samples (Table 5.5) was

higher in Aries HD than in Yatsyn 1 pasture during the intake measurement in March (73.6 vs 65.6 ± 1.27 %, $P = 0.0818$). Wrightson Seeds Ltd have demonstrated higher levels of organic matter digestibility in Aries HD than in Yatsyn 1 pastures (87.2 vs 84.6) cut to grazing height every four weeks from November to February and allowed to regrow (Appendix 1.1). To simulate these conditions, herbage was cut under cages using the same management. From November to February (12 samples/cultivar) the organic matter digestibility was 79.4 vs $77.6 \pm 2.37\%$ ($P = 0.3968$) for Aries HD and Yatsyn 1 pasture respectively. *Poa annua* was a major weed contaminant in the pasture and may have diluted contrasts in digestibility between cultivars.

In the previous weaned lamb finishing experiment (Chapter 3) lambs gained 104 vs 84 g/day on Aries HD and Yatsyn 1 respectively. The better animal performance achieved on Aries HD pasture was attributed to the effects of alkaloid concentrations and associated ryegrass staggers. Although lolitrem B levels were the same between cultivars (0.96 ppm), ergovaline concentration was twice as much in Yatsyn 1 herbage as in Aries HD (0.42 vs 0.23 ppm, $P < 0.05$). Incidence of staggers was twice as high on Yatsyn 1 than on Aries HD pasture (29 vs 15% lambs severely affected, $P < 0.05$).

There was no significant difference in animal performance measured by liveweight gain, carcass weight gain and wool production between Aries HD and Yatsyn 1. A mean chromium release rate of 141 mg/day was used to calculate intake. There was also no significant difference in herbage intake measured in January and in March. In actual terms, estimates were low compared to predictions made based on energy requirements (1.4 kg OM/head/day; Geenty and Rattray 1987). Possible reasons for low estimates were discussed in Chapter 4. However, estimates were useful for a relative comparison between cultivars.

Lolitrem B is the main alkaloid responsible for causing ryegrass staggers (Gallagher et al. 1981). In this experiment lolitrem B levels were lower than in the 1995/96 season and did not exceed 1.4 ppm (Figure 5.2). A lolitrem B concentration of 2.0 ppm is required for the development of clinical ryegrass staggers (di Menna et al. 1992; Blythe

et al. 1993). High lolitrem B concentrations often accompanying a period of elevated temperatures and minimal rainfall (Hawkes et al. 1995). In this experiment monthly mean rainfall was consistent and maximum air temperatures were not high. In addition pastures were sprinkler-irrigated in January through to March to promote green vegetative growth and staggers rarely occurs on irrigated pastures (Fletcher and Piper 1990). The lambs therefore had a lower alkaloid challenge than in the previous summer, and this was reflected in the low incidence of staggers.

The ergopeptine alkaloids and in particular ergovaline can cause hyperthermia and depressed serum prolactin levels in sheep during summer (Fletcher and Barrell 1984; Fletcher 1993b). In this study there was no difference in rectal temperature and serum prolactin concentration between lambs grazing the two cultivars. However, without a nil-endophyte treatment it was not possible to discern the normal rectal temperature and prolactin concentration in the lambs. For sheep, there is often little or no difference in serum prolactin levels at ambient temperatures below 22 °C (Fletcher and Easton 1997). Ambient temperatures did not exceed 22 °C on measurement days and ergovaline levels were undetectable so it was unlikely that sheep were affected by heat stress in this study.

5.6 CONCLUSIONS

The nutritive value and endophyte alkaloid concentrations were similar between Aries HD and Yatsyn 1 cultivars. This resulted in no difference in feeding value between the two cultivars over the summer/autumn period as measured by the performance of weaned lambs.

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CHAPTER 6

SURVIVAL, REPRODUCTIVE DEVELOPMENT AND DENSITY OF TILLERS IN PURE SWARDS OF ARIES HD AND YATSYN 1 PERENNIAL RYEGRASS (*Lolium perenne*)

6.1 ABSTRACT

Aries HD perennial ryegrass was bred specifically for improved digestibility over the summer and autumn period (November to February). A tiller demography experiment was conducted to compare the patterns of reproductive development and tiller survival between Aries HD and a control cultivar (Yatsyn 1) perennial ryegrass. Clover-free swards were established in the autumn of 1995, in a randomised block design with three replicates of each cultivar of 0.33 ha which were continuously grazed with sheep. Each replicate had five transects randomly placed with 10 identified tillers and tiller survival and reproductive development was recorded at weekly intervals from 4 September 1996 to 26 March 1997. Few tillers died until mid December, after which survival approximated an exponential decay curve. There was no difference in the rate of tiller death between Aries HD and Yatsyn 1 swards. Seedheads were first observed on 19 November and were still visible on 26 March. Aries HD appeared to have a greater proportion of initial reproductive tillers, but after January a lower proportion than Yatsyn 1. Mean tiller density increased from October (2950 tillers/m²) to January (5010 tillers/m²), declining again in March (3160 tillers/m²), and there were no significant differences between cultivars. Yatsyn 1 tillers were consistently heavier than Aries HD tillers but this difference was not significant (8.5 vs 7.4 ± 0.90, P = 0.2685).

This chapter forms the basis of a paper for submission to: *The Proceedings of the New Zealand Grassland Association.*

6.2 INTRODUCTION

Aries HD perennial ryegrass was bred specifically for improved whole plant digestibility over the summer and autumn period (November to February). The breeding program began in 1986 when selections of Waikato plants were taken. In 1988 a total of 50,000 single plants were sown. In 1989 a selection of 525 elite families were sown at four sites (Canterbury, Waikato, Victoria and in Europe). In the autumn of 1990 samples from these plants were analysed for forage quality and minerals using NIRS analysis. In the winter of 1990 agronomic, quality and mineral data was pooled. Seed was harvested from 7 elite clones to form Aries HD perennial ryegrass in 1990/91. Since then, Wrightsons have demonstrated an organic matter digestibility advantage of 2.6% over Yatsyn 1 using cutting management (Appendix 1.1). Since organic matter digestibility integrates all components of digestibility such as leaf to stem ratio and the proportion of cell and structural components of the plant, it was not yet known which component had been altered.

Significant differences in digestibility between ryegrass progenies over maturity may be due to differences in earliness of flowering (Dennis and Frandsen 1986). At the same calendar date, a later flowering cultivar would have a higher digestibility and leaf to stem ratio because of their slower rate of reproductive development (Van Wijk et al. 1993; Vogel and Sleper 1994). In Chapter 4 it was shown that proportion of leaf was significantly greater in Aries HD than in Yatsyn 1 pasture over September to November, perhaps reflecting a different rate of tiller maturity. Ewes achieved significantly better liveweight gain on Aries HD pastures than on Yatsyn 1 pastures, resulting from a higher herbage intake and bite rate. However, the greater leaf content of the Aries HD pasture was not reflected in a higher digestibility. In summer, leafy vegetative swards are preferable to stemmy swards with a large proportion of dead herbage, because of their greater herbage production and quality (Korte et al. 1984). Cultivar differences in reproductive growth can result from differences in the proportions of vernalised tillers, the timing of stem elongation, or in the growth rates of reproductive tillers (Hunt and Mortimer 1982). Information is presented here on the relative rates of reproductive

development between the two cultivars. Because reproductive tillers die when they reach maturity, the rates of tiller death were also recorded (Woodward 1998).

6.3 MATERIALS AND METHODS

6.3.1 Experimental site and duration

The experiment was conducted on the Sheep and Beef Cattle Research Unit, Massey University, Palmerston North. Clover-free swards of Aries HD and Yatsyn 1 were established in the autumn of 1995, in a randomised complete block design with three blocks (0.33 ha/plot). Measurements of ryegrass tillers (150 per treatment) began on 4 September 1996 and finished on 26 March 1997, and so spanned both the lactation (Chapter 4) and the weaned lamb experiments (Chapter 5).

6.3.2 Tiller demography

Tiller dynamics was observed under continuous grazing conditions. Five transects, each 1-m long, were randomly placed in each plot. Wooden pegs were placed at each end to aid relocation of transects (Plate 6.1). Ten tillers spaced at 10-cm interval along each transect were identified with a brightly coloured plastic tie, and tiller survival and reproductive development was recorded at weekly intervals. Vegetative tillers were classed as dead when they were brown and withered, and reproductive tillers were classified as dead when the defoliated stem was brown and sapless (Korte et al. 1985).

Data calculation

Tiller survival/longevity was expressed as the ratio of surviving tillers to the initial number of tagged tillers, using equation (1) (Korte 1986).

$$\text{Survival} = N_t / N_0 - m \quad (1)$$

where $N_0 - m$ was the initial number of tillers (50/plot) corrected for the number of missing tillers on that measurement day and N_t the number of tillers alive on that measurement day.

Reproductive development was expressed as the ratio of flowering tillers (with a visible seedhead) to the initial number of tillers at the beginning of the experiment, corrected using equation (1). Reproductive development was also expressed as the ratio of newly elongating tillers or newly flowering tillers to the number of live tillers from the previous measurement date, using equation (2).

$$\text{Reproductive development} = N_{r(t-1)} / N_{(t)} - m \quad (2)$$

Where $N_{(t)} - m$ was the number of live tillers from the previous measurement day corrected for missing tillers and $N_{r(t-1)}$ the number of newly elongating or flowering tillers.

6.3.3 Tiller population density and tiller weight

Tiller population density was calculated from 40 pasture cores per plot, (October, January and March) each 50 mm in diameter, taken at random, and the number of ryegrass and other species (predominantly *Poa annua*) tillers/core recorded (Mitchell and Glenday 1958). Ryegrass tillers from cores were bulked, oven-dried at 80 °C for 24 hours and weighed to determine mean tiller dryweight. A tiller corer for taking soil core samples is shown in Plate 6.2.

6.3.4 Statistical analysis

Analysis of variance and repeated measures analysis were carried out on plot mean values with three replicates, using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc. 1988).

Plate 6.1 Transect with 10 cm intervals marked along a ruler.



Plate 6.2 Tiller corer for taking soil core samples for the measurement of tiller population density.



6.4 RESULTS

6.4.1 Tiller survival

Survival of tillers tagged at the start of the experiment (4 September 1996) is shown in Figure 6.1. Few tillers died until about 11 December 1996, after which survival approximated an exponential decay curve (Figure 6.2). The exponential decay model (e^{bt}) was a good fit for to data, and the mean curve for both cultivars explained 97% of the variation in tiller survival from this data. Regression coefficients and coefficients of determination (R^2) for this model are given in Table 6.1. The constant (b) is the rate of tiller death. Constant b was converted to a half-life ($t_{1/2}$), the time taken for half the tillers to die as defined by Korte (1986). There was no significant difference between rate of tiller death (b) between Aries HD and Yatsyn 1 swards (Table 6.1).

Figure 6.1 Survival of tillers from September to March 1997. The surviving number of tillers is expresses as the proportion $N_t/N_0 - m$ (Equation 1) for Aries HD (◆) and Yatsyn 1 (■).

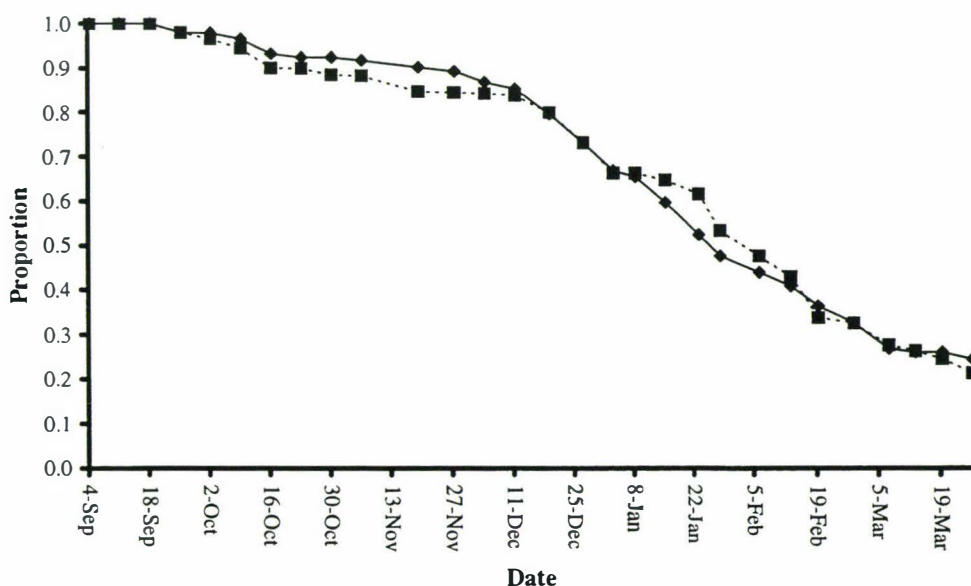


Figure 6.2 Survival of tillers from December to March 1997. The surviving number of tillers is expressed as the proportion $N_t/N_0 - m$. The fitted line (e^{bt}) has $b=-0.0133$, the mean of Aries HD (◆) and Yatsyn 1 (■).

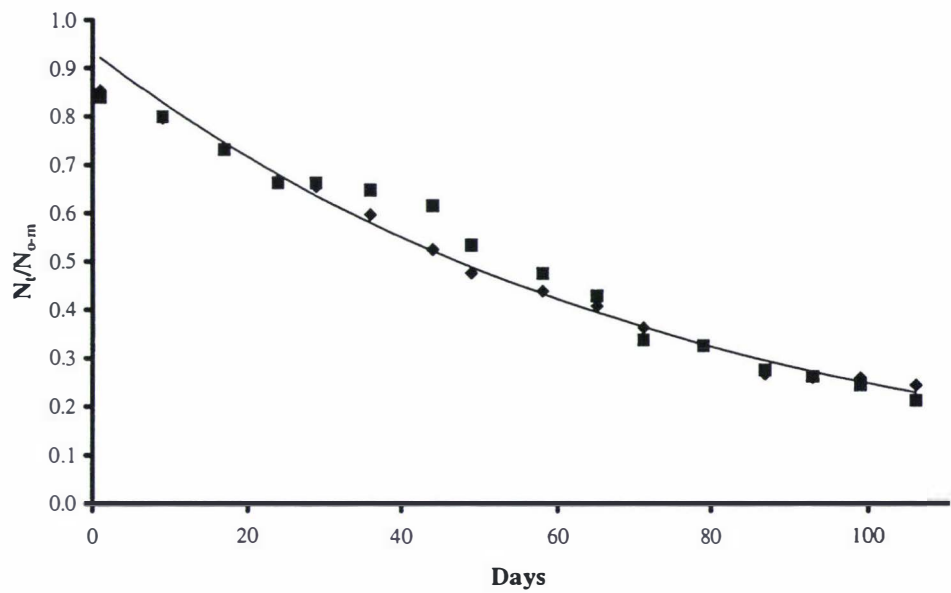


Table 6.1 Rate of tiller death (regression coefficient, b), coefficients of determination (R^2), and half-life ($T_{1/2}$) of tillers for Aries HD and Yatsyn 1 cultivars.

Cultivar	Rate of tiller death (b)	Half life ($T_{1/2}$)	R^2
Aries HD	-0.0129	53	0.909
Yatsyn 1	-0.0136	51	0.959
Mean	-0.0133	52	0.986
SEM	0.00162	-	-
P	0.8679	-	-

6.4.2 Reproductive development

The proportion of tillers with a seedhead, expressed as a proportion of tagged tillers at the start of the experiment, is shown in Figure 6.3. Tillers with a visible seed head were first observed on the 19/11/96 and were noted until recording ceased on the 26/3/97. The mean proportion is given in Table 6.2. Aries HD appeared to have a greater proportion of flowering tillers in December and then a lower proportion than Yatsyn 1 swards after January. No difference was statistically significant (Table 6.2). The development of newly flowering tillers expressed as a proportion of potential tillers that could become reproductive (number of live tillers) from the previous measurement day is shown in Figure 6.4. Newly elongating tillers expressed in the same way is shown in Figure 6.5. No significant difference between cultivars in either stem elongation or seedhead development was detected (Table 6.2). The total percentage of tillers tagged at the start of the experiment that subsequently was observed elongating was 62% in both Aries HD and Yatsyn 1 swards (Table 6.2). The total percentage of tillers which was observed with a seedhead was 24% in both Aries HD and Yatsyn 1 swards.

Figure 6.3 Proportion of tillers with a seedhead, expressed as the proportion $N_t/N_0 - m$ (Equation 1) for Aries HD (◆) and Yatsyn 1 (■).

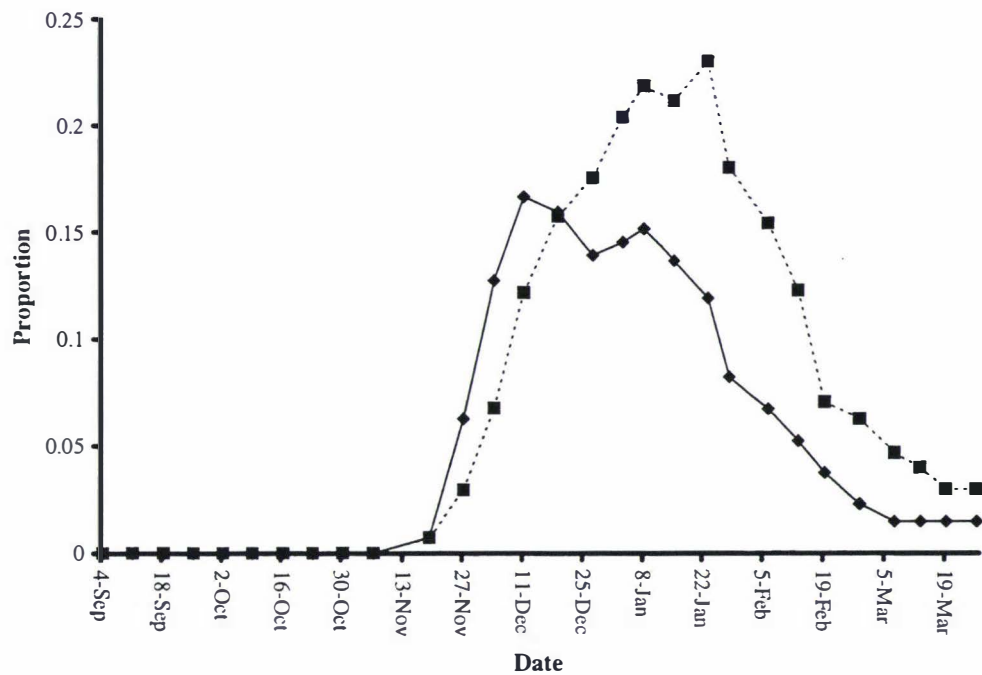


Figure 6.4 Seedhead development, expressed as the proportion of newly flowering tillers to the number of live tillers from the previous measurement date (Equation 2) for Aries HD (◆) and Yatsyn 1 (■).

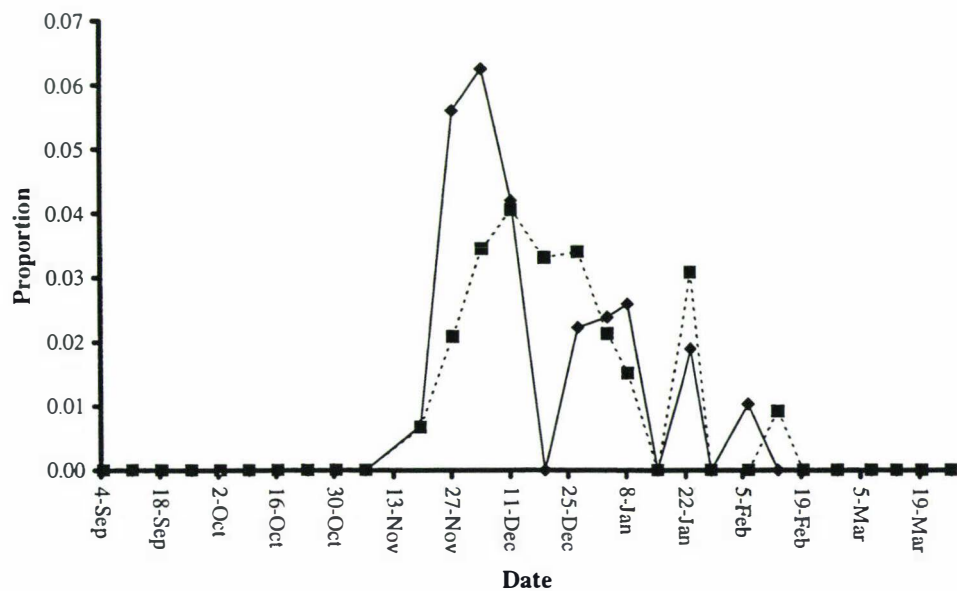


Figure 6.5 Stem elongation, expressed as the proportion of newly elongating tillers to the number of live tillers from the previous measurement date (Equation 2), for Aries HD (◆) and Yatsyn 1 (■).

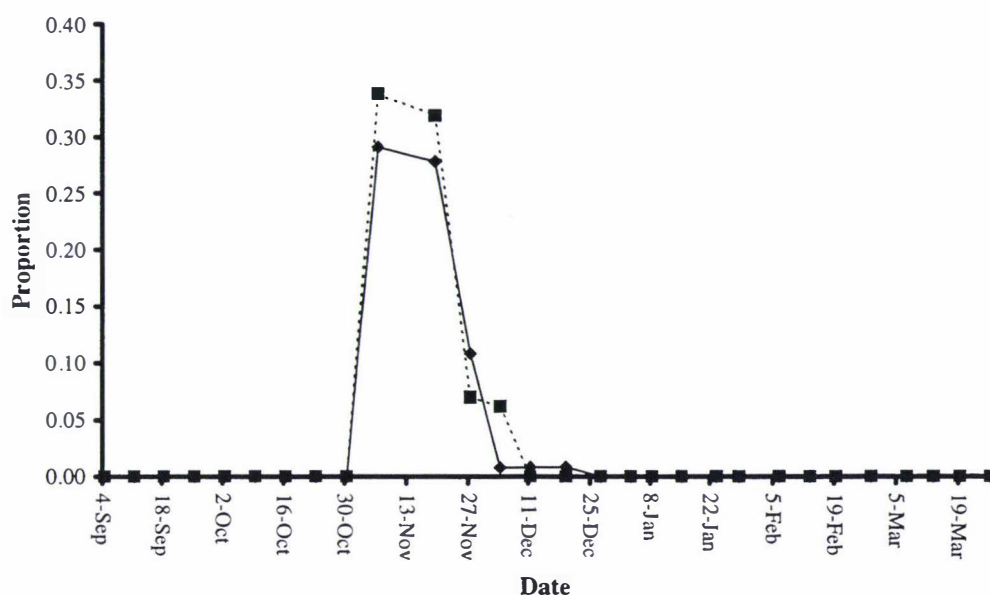


Table 6.2 Summary of flowering behaviour in populations of tillers of Aries HD and Yatsyn 1.

Ratio	Aries HD	Yatsyn 1	SEM	P
¹ Proportion of flowering tillers to initial number of tillers	0.081	0.114	0.1007	0.4202
² Proportion of newly elongated tillers to the number of live tillers	0.027	0.040	0.0263	0.3284
³ Proportion of newly flowering tillers to the number of live tillers	0.122	0.154	0.0245	0.1586
Total percent observed elongating	62.0	62.7	6.18	0.9462
Total percent with a visible seedhead	24.0	23.3	3.77	0.9120

1 Mean of 19 measurement dates between 19/11/96 to 26/3/97 (Equation 1)

2 Mean of 6 measurement dates between 6/11/96 to 19/12/96 (Equation 2)

3 Mean of 13 measurements dates between 19/11/96 to 13/2/97 (Equation 2)

6.4.3 Tiller population density and tiller weight

Ryegrass tiller population density (Figure 6.6) increased from October (3158 vs 2731 \pm 275 tillers/m², $P < 0.3876$ for Aries HD and Yatsyn 1 respectively) to January (5034 vs 4977 \pm 866 tillers/m², $P < 0.9670$ for Aries HD and Yatsyn 1 respectively). Population density declined again in March to 3075 vs 3237 \pm 250 tillers/m² ($P < 0.6909$) for Aries HD and Yatsyn 1 respectively. Change in tiller density over time was significant ($P < 0.0416$). *Poa annua* was a major (but similar) weed contaminant in both cultivars. Yatsyn 1 tillers were consistently heavier than Aries HD tillers (Figure 6.7) although this difference was statistically insignificant (mean over three dates, 8.5 vs 7.4 \pm 0.90, $P < 0.2685$).

Figure 6.6 Tiller population density of Aries HD and Yatsyn 1.

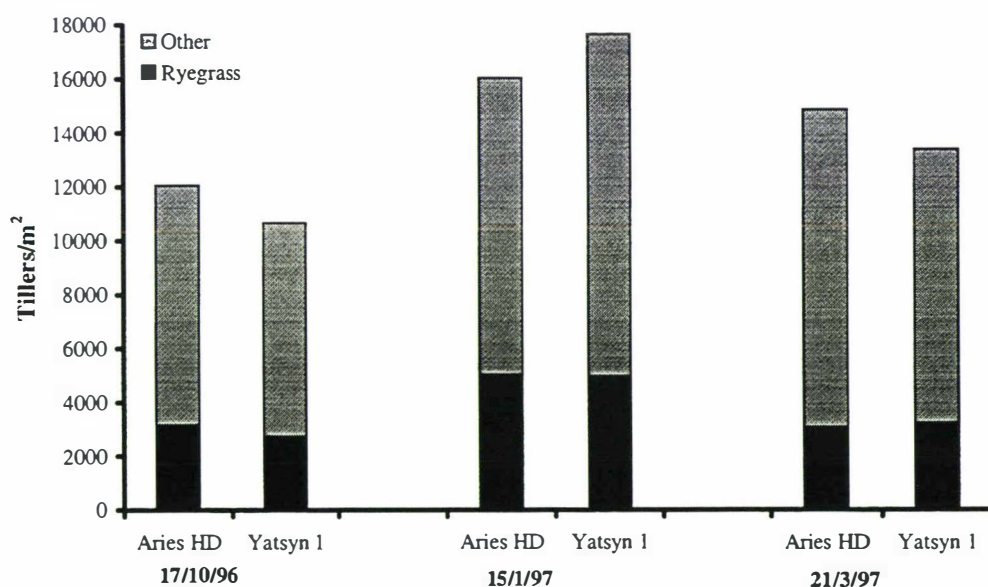
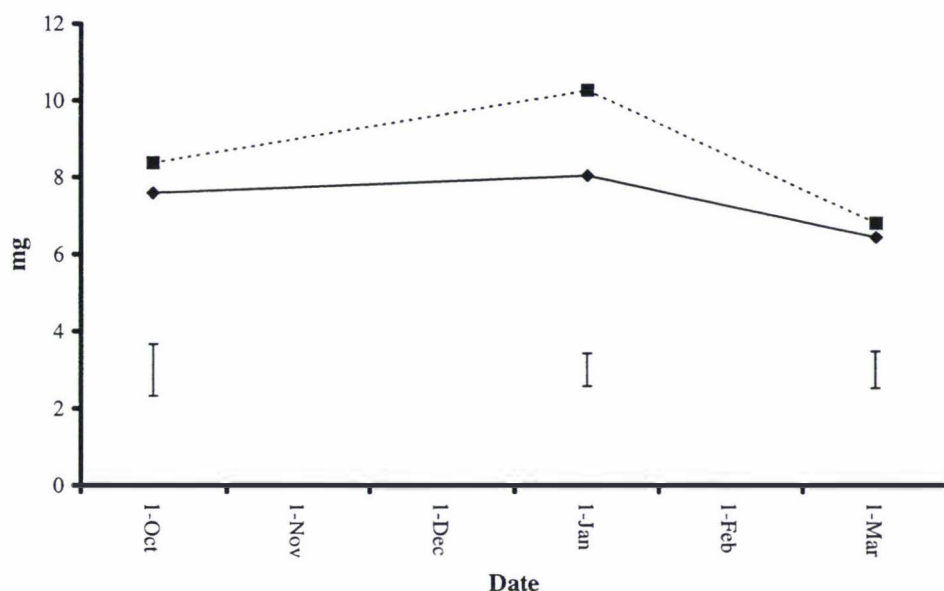


Figure 6.7 Ryegrass tiller weight for in Aries HD (◆) and Yatsyn 1 (■) swards. Bars indicate SED 5%.



6.5 DISCUSSION

The main objective of this experiment was to determine if there were any differences in the survival of tillers and in the onset, duration and intensity of reproductive development between Aries HD and Yatsyn 1 cultivars. Observations on tiller behaviour were made under continuous sheep grazing. This meant that reproductive tillers may have been defoliated before they reached the seedhead stage so were not included in this category. We assumed that the probability of tillers being grazed was a random effect and equal between plots. Parsons et al. (1984) has reported marked differences in the structure and physiology of tillers between continuously grazed swards compared with in ungrazed swards. In a comparison of caged and ungrazed tillers, Grant et al. (1989) found that 30% of larger than average tillers were lost due to grazing. It was also reported that senescence rates were higher in grazed plots than under cages, with the conclusion that it was more important to avoid the bias associated

with the environmental effects of caging and cessation of grazing, than avoid loss of tillers to grazing (Grant et al. 1989).

There were no obvious differences in tiller death rate between the two cultivars. Aries HD and Yatsyn 1 cultivars had similar tiller survival patterns to Grasslands Nui (Korte 1986). Korte showed that tiller survival approximated an exponential decay curve and has regression coefficients (b) and half-life estimates ($b=-0.0124$ and $t_{1/2}=56$ days for summer 1978) were similar to those calculated for Aries HD and Yatsyn 1 tillers.

Cultivar differences in reproductive growth can result from differences in the proportions of vernalised tillers, the timing of stem elongation, or in the growth rates of reproductive tillers (Hunt and Mortimer 1982). Figure 6.3 shows an apparent difference between cultivars in the proportion of flowering tillers over summer. However, Figure 6.3 only represents the proportion of the original number of tagged tillers existing with a seedhead and does not account for the increasing loss of tillers due to death and grazing. Therefore, Figures 6.4 and 6.5 were produced to show only newly reproductive tillers expressed as the proportion of tillers potentially able to flower (live tillers only).

Stem elongation was first observed on 6 November with the first seedheads observed two weeks later on the 19 November. Aries HD appeared to have a more rapid onset of inflorescence and then a lower proportion after initial flowering than Yatsyn 1. Korte et al. (1984) observed that most Nui reproductive tillers (2400 m^{-2}) appeared in an initial group, with a secondary group of reproductive tillers (1000 m^{-2}) appearing during November and December. Culms on secondary reproductive tillers did not develop until the initial primary tillers have been defoliated, explaining why secondary reproductive tillers did not appear in the ungrazed control swards. He suggested (Korte 1986) that these secondary reproductive tillers might have originated from vernalised tiller buds that had previously failed to express because of apical dominance. Aries HD is thought to have lower aftermath heading than Yatsyn 1 (M. N. Norriss, personal communication) which is supported to a limited extent by the data in this study. Aries HD appeared to have a lower proportion of tillers elongating than Yatsyn 1. However,

the total proportion of tillers becoming reproductive (visible seedhead) was 24% in both Aries HD and Yatsyn 1 ryegrass.

It was found in Chapter 4 that the percentage of leaf was significantly higher in Aries HD than in Yatsyn 1 swards from September to November. Evidence here shows that Aries HD began flowering slightly earlier than Yatsyn 1 (Figure 6.4) suggesting that the difference in leaf content was not due Aries HD being slower developing than Yatsyn 1. There was no difference in the percentage of leaf over December to March (Chapter 5), when lamb performance was the same between the two cultivars. *In vitro* organic matter digestibility, neutral detergent fibre or nitrogen content did not differ between cultivars at any stage during this experiment.

Aries HD had slightly finer tillers (15%) and on two dates had a higher tiller density. However these differences were statistically non-significant. In a comparison under intensive sheep grazing it was observed that Grasslands Nui had heavier tillers (50%) and more leaves per tiller (7%) than Grasslands Ariki which had more tillers per plant (25%). Because the difference in tiller number and weight was offset there was little difference in plant dry weight. There was no difference in the population structure between Nui and Ariki. Hunt and Mortimer (1982) found that monocultures of Nui had larger (43%) but less dense (20%) tillers than monocultures of Grasslands Ruanui. This observation is known as tiller/density compensation, and implies that when individual tillers or shoots are larger the population density is correspondingly decreased (Matthew et al. 1996).

6.6 CONCLUSIONS

Aries HD and Yatsyn 1 cultivars had similar tiller survival patterns to those reported for Grasslands Nui in the literature. Aries HD appeared to have a more rapid onset of initial flowering but then a lower proportion of flowering from secondary reproductive tillers. This contrast was non-significant and did not result in any contrasts in herbage nutritive value during this experiment. There is an indication that Aries HD swards have a higher density of finer tillers than Yatsyn 1.

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CHAPTER 7

EXPERIMENT 4: PERFORMANCE OF LAMBS AND THE INCIDENCE OF STAGGERS AND HEAT STRESS ON TWO PERENNIAL RYEGRASS (*Lolium perenne*) CULTIVARS USING A LEADER-FOLLOWER ROTATIONAL GRAZING MANAGEMENT SYSTEM

7.1 ABSTRACT

A summer lamb finishing experiment was conducted at Palmerston North, New Zealand to compare animal performance and health of weaned lambs grazing Aries HD and Yatsyn 1 perennial ryegrass (*Lolium perenne*). Aries HD ryegrass has been selected for increased digestibility in summer and early autumn. Lambs were rotationally grazed in a leader-follower sequence on each cultivar to assess both the nutritive value of the pastures and the alkaloid challenge imposed by the respective endophyte (*Neotyphodium lolii*)/cultivar associations. Sixty lambs were used in the experiment (15 per cultivar/grazing sequence). The leader-follower regime created differences in pre-grazing sward height (13.5 vs 7.0 ± 1.00 cm, $P < 0.0001$), herbage mass (4400 vs 3800 ± 220.8 kg DM/ha, $P < 0.01$) and botanical composition between leader and follower pastures, respectively. There were higher proportions of ryegrass (60 vs $47 \pm 2.7\%$, $P < 0.0001$) and lower proportions of dead material (35 vs $48 \pm 3.4\%$, $P < 0.001$) in leader pastures. Leader pastures were higher in *in vitro* organic matter digestibility (62 vs $55 \pm 2.4\%$, $P < 0.005$) and lower in neutral detergent fibre (58 vs $63 \pm 1.5\%$, $P < 0.001$) than follower pastures. There were significantly faster liveweight gains (92 vs 53 ± 10.6 g/day, $P < 0.0005$) in leader lambs than in follower lambs. Ryegrass cultivars did not differ in the sward components, organic matter digestibility, neutral detergent fibre, nitrogen content or liveweight gain. Lambs grazing Aries HD pasture had higher herbage intakes in late January than those grazing Yatsyn 1 pastures (0.95 vs $0.85 \pm$

0.023 kg DM/day, $P < 0.005$). Final lamb liveweight was 35.2 vs 30.5 ± 0.56 kg for leader and follower lambs respectively ($P < 0.0001$).

Management and cultivar did not affect the concentrations of lolitrem B or peramine in herbage samples. Ergovaline concentration was significantly lower in Aries HD than in Yatsyn 1 pasture samples (0.25 vs 0.49 ± 0.050 ppm, $P < 0.0001$). Serum prolactin concentration was reduced in lambs grazing follower swards (181 vs 120 ± 18.0 ng/ml). Incidence of ryegrass staggers was 100% in all treatments from 10 February to 9 March when lambs were removed. Both grazing sequence and cultivar significantly affected severity of staggers. Staggers score was highest in Yatsyn 1 followers (4.4) and lowest in Aries HD leaders (3.1). Respiration rate was higher in leaders than in followers (77 vs 75 ± 1.1 breaths/minute, $P < 0.001$), and in Yatsyn 1 than in Aries HD lambs (77 vs 74 ± 1.1 breaths/minute, $P = 0.06$). There was no difference in faecal moisture between treatments but a significant difference in faecal contamination score (dags) between leaders and followers (1.7 vs 1.1 ± 0.33 , $P < 0.05$). It was concluded that grazing management and cultivar affected the alkaloid challenge of lambs, resulting in differences in ryegrass staggers severity, heat stress and performance of weaned lambs grazing ryegrass over the summer and autumn.

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7.2 INTRODUCTION

Approximately 7 million hectares in New Zealand is sown with perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens*) and is the basis for the majority of the sheep, beef and dairy production (Fletcher 1993a). The majority of this ryegrass contains endophyte (*Neotyphodium lolii*; Glenn, Bacon & Hanlin = *Acremonium lolii*). Perennial ryegrass containing endophyte is now generally preferred by the pastoral industry in New Zealand because it is more persistent under threat from drought and pasture pests (Fletcher and Piper 1990). *Neotyphodium lolii* protects against attack by Argentine stem weevil (*Listronotus bonariensis*) which is one of New Zealand's major insect pests (Rowan and Gaynor 1986). However, some alkaloids produced from the association when present in sufficiently high levels can adversely affect the health and production of grazing animals (Latch 1994). Endophyte effects on grazing animals include ryegrass staggers, reduced liveweight gains, increased faecal moisture (scouring) and faecal soiling (dags), increased incidence of flystrike, increased rectal temperatures and respiration rates, and depressed serum prolactin levels (Fletcher 1993a; Familton et al. 1995; Fletcher et al. 1996).

The alkaloid lolitrem B and possibly other lolitrems cause ryegrass staggers (Gallagher et al. 1981). Affected animals show in-co-ordination, hypersensitivity to external stimuli and later tetanic muscle spasms (Rowan 1993). The ergopeptide alkaloids and in particular ergovaline depress basal prolactin levels in grazing animals and are also considered to be responsible for the hyperthermia reported in sheep during summer (Fletcher and Barrell 1984; Piper and Fletcher 1990; Fletcher et al. 1990; Rowan et al. 1990; Fletcher 1993a, Fletcher et al. 1993b). In New Zealand losses associated with *Neotyphodium lolii* infected ryegrass have been estimated to be in excess of \$100 million dollars (Familton et al. 1995). All the alkaloids produced by ryegrass/endophyte associations have a distinct seasonal profile with concentrations being highest in summer and autumn, falling to minimal levels in winter and early spring (Fletcher et al. 1996).

The strain of endophyte and the host ryegrass cultivar can determine the presence and quantity of the alkaloids lolitrem B, peramine and ergovaline produced and animal response (Barker et al. 1993; Davies et al. 1993; Garthwaite et al. 1993; Fletcher and Sutherland 1993; Powell et al. 1993). Endophyte is an added dimension in assessing animal production from perennial ryegrass. When endophyte is combined with a perennial ryegrass cultivar with high genetic quality factors the detrimental effects of lower liveweight gains may be reduced (Fletcher 1986). One way of overcoming the undesirable properties of endophytes is to seek ryegrass/endophyte associations that do not produce compounds harmful to animals (Latch 1994).

Preliminary work by Wrightson Seeds (Appendix 7.1) and in Experiment 1 (Chapter 3) indicated that Aries HD perennial ryegrass with wild-type endophyte produces half as much ergovaline as Yatsyn 1 perennial ryegrass with wild-type endophyte (0.23 vs 0.70; $0.23 \text{ vs } 0.42 \text{ ppm} \pm 0.042 \text{ ppm}$, $P < 0.026$). Ergovaline is a dopamine agonist that can act as a vaso-constrictor, and can reduce peripheral blood flow and the ability to dissipate excess heat (Fletcher 1993b). Low levels of ergovaline have been found to provoke maximum depression of prolactin in sheep (Cheeke et al. 1993). This experiment was designed to compare the nutritive value (organic matter digestibility, neutral detergent fibre and nitrogen percentage) and the concentrations of alkaloids produced (lolitrem B, ergovaline and peramine) by Aries HD and Yatsyn 1 perennial ryegrass, and relate them to lamb health (ryegrass staggers, heat stress) and performance (liveweight gain).

7.3 MATERIALS AND METHODS

7.3.1 Experimental design

A summer lamb finishing experiment with 60 weaned lambs was conducted at Massey University Pasture and Crop Research Unit, Palmerston North (40°23'S), from 2 December to 7 April 1998. The experimental site was located on a Tokomaru silt loam classified as an Argillic-Fragic, Perch-grey, Pallic soil (Hewitt 1992).

The experiment was designed as a 2 x 2 factorial, with two perennial ryegrass cultivars (Aries HD and Yatsyn 1) and two grazing sequences (Appendix 7.2). Two groups of lambs were rotationally grazed on each cultivar in a leader/follower sequence.

7.3.2 Pastures

Clover-free swards of Aries HD and Yatsyn 1 perennial ryegrass were established in the autumn of 1997 using conventional cultivation. There were six replicate plots (0.2 ha) of each cultivar arranged in a randomised block design. Three applications of 60 kg/ha of urea were made in November, December and January after grazing, corresponding to 28.8 kg N/ha. In December, plots were sprayed after grazing with the herbicides Tordon 50-D (picloram and 2, 4-D; Dow Elanco, NZ Ltd) at 4 l/ha to control broadleaf weeds and Nortron (Ethofumesate; BASF, NZ Ltd) at 4 l/ha to control *Poa annua*.

7.3.3 Animals

A total of 75 Suffolk x Romney lambs with mean initial liveweight 25.9 ± 0.56 kg were used in this experiment. The lambs were allocated in equal numbers to five groups balanced for sex and initial liveweight. One group was slaughtered at the start of the experiment to provide a basis for predicting the initial carcass weight of the 60 remaining lambs. The other four groups of lambs were allocated to the four (two cultivars, two grazing sequences) treatments.

All lambs were ear-tagged according to treatment group and drenched at monthly intervals with cydectin (moxidectin; Cyanamid of New Zealand) to removed internal parasites. The lambs had free access to water.

7.3.4 Grazing management

Pairs of plots of each cultivar were grazed in a rotation. Each leader group of 15 lambs grazed each plot in sequence for seven days, followed by the follower group of 15 lambs for seven days. The leaders grazed fresh regrowth (42 day rotation and 28 days

regrowth between grazings) while the followers grazed the residue left behind by the leaders. This leader/follower grazing management created a contrast in herbage quantity and nutritive value, and in the concentration of endophyte alkaloids available to the lambs. It was designed to separate contrasts in nutritive value from endophyte alkaloid levels produced by the two cultivars. It was anticipated that the leader lambs would test the nutritional value of the pasture, while the follower lambs were forced to graze into the base of the sward possessing the greatest potential for endophyte toxicity.

7.3.5 Pasture measurements

Herbage mass (kg DM/ha) was measured before leader lambs were shifted into a new plot, immediately after the leaders left, and after the followers left. The pre-grazing herbage mass of the follower group was also the post-grazing mass of the leader group. Herbage mass was determined by cutting eight rectangular 0.1 m² quadrats per plot to soil level using an electric shearing hand-piece. The herbage samples were then washed, oven-dried at 80 °C for 24 hours and weighed individually. Sward surface height was also measured before and after grazing, from fifty readings taken at random with a sward stick on each occasion (Barthram 1986).

Botanical composition was determined from bulked samples cut to ground level at the same time as herbage mass was estimated. The samples were separated into ryegrass leaf and stem, other species and dead material. A sample of 300 ryegrass tillers were recorded as vegetative or reproductive (exhibiting stem elongation), and the proportion of vegetative tillers calculated. Tiller population density was calculated from 40 pasture cores per plot, each 50 mm in diameter, taken at random before the leader group commenced grazing, and the number of ryegrass and other species tillers/core recorded (Mitchell and Glenday 1958). Endophyte infection was determined using ten tillers/plot on 27/3/97 and examining stained epidermal strips from leaf sheaths under a light microscope (Latch and Christensen, 1985).

For laboratory analysis, samples of herbage on offer were cut to soil level from each plot when the lambs were introduced. Two 0.33 x 0.67 m areas of pasture were

protected from grazing by wire mesh cages placed in each plot. At the end of grazing the cages were removed and samples cut to grazing height (herbage selected). Herbage samples were stored at -20°C , freeze-dried and ground to pass a 1-mm mesh diameter sieve before laboratory analysis (see 7.3.7).

7.3.6 Animal measurements

All lambs were weighed at fortnightly intervals at the end of the grazing week (Plate 7.1). Lambs were scored for severity of ryegrass staggers on a 0 – 5 scale (0 = no visible symptoms; 5 = unable to walk from the paddock, Appendix 2.1). Staggers was assessed weekly during the experiment. Lambs unable to walk to weighing were transported. Lambs were scored for faecal dags every fortnight. This was a visual assessment on a 0 (no dags) to 5 (severe dags) scale. Faecal moisture was also assessed in January and February. Faecal grab samples were collected from each individual lamb in a plastic pot and weighed. Samples were dried at 80°C for 72 hours, weighed and faecal moisture percentage calculated.

On six occasions (16 January to 17 February) lambs were housed in an enclosed shed to monitor heat stress symptoms. All lambs were brought to the shed and groups of twelve lambs were randomly chosen and minimally restrained in specially constructed herringbone type bails during sampling (Plate 7.2). Ambient temperature inside the enclosed shed was recorded every half-hour. Rectal temperature was recorded using digital clinical thermometers. Respiration rates were measured by counting the number of respirations in 30 seconds using a timer with an audible alarm. Blood samples were drawn on 3, 4, 11 and 13 February by jugular venipuncture (Plate 7.2) into EDTA vacutainers and assayed for serum prolactin using a radioimmunoassay technique (Kirkwood et al., 1984). All lambs remained inside the shed for a maximum of 1.5 hours before being returned to the field.

Plate 7.1 Recording liveweight of lambs in Experiment 4.



Plate 7.2 Drawing blood sample using jugular venipuncture for analysis of serum prolactin concentration. Bails used for restraining lambs for measurement of rectal temperature and respiration rate are in the background.



Herbage intake was measured in January 1998. Faecal organic matter output was estimated using intra-ruminal slow release chromium sesquioxide capsules (CRD, Cr₂O₃ matrix, Captech New Zealand Ltd, Auckland, NZ) as described by Parker et al. (1989). Chromium capsules were orally administered to each lamb on 12 January. Following an eight day acclimatisation period, faecal grab samples were collected in plastic bottles, for two consecutive four-day periods. They were oven dried at 80 °C for 72 hours, and bulked across days for each animal on an equal dry weight basis within each four-day period. Samples were ground and stored until required for laboratory analysis. Five sheep fistulated in the oesophagus were rotated among the swards, to collect samples of the herbage selected for estimation of *in vitro* organic matter digestibility (Roughan and Holland 1977). The fistulates were switched between leader and follower groups every day. One sample was taken from each fistulate within each treatment during each period; thus twenty samples of extrusa were collected in each four-day period. The extrusa samples were immediately placed in an icebox, frozen and stored at -20°C. They were then freeze-dried, and ground to pass through a 1-mm mesh screen before laboratory analysis. Chromium release rate was estimated from chromium capsules recovered at slaughter from the previous lamb finishing experiment (Chapter 5).

7.3.7 Laboratory analysis

Samples of herbage on offer and herbage selected were analysed for *in vitro* organic matter digestibility using the enzymic method developed by Roughan & Holland (1977). Herbage on offer was analysed for nitrogen by the Kjeldahl procedure and neutral detergent fibre by the detergent system of Van Soest (1994). Chromium analysis of faeces following the method of Costigan and Ellis (1987). Samples of herbage on offer were analysed for concentrations of lolitrem B, peramine and ergovaline by HPLC (Barker et al. 1993).

7.3.8 Statistical analysis

Lamb liveweight, herbage intake, staggers severity, respiration rate, rectal temperature, faecal dag score and serum prolactin concentration were analysed using repeated

measures analysis in the General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 1987). The model was a 2 x 2 factorial design, with two perennial ryegrass cultivars (Aries HD and Yatsyn 1) and two grazing sequences (leaders and followers). Lamb liveweight gain, herbage intake, and indicators of animal health, were analysed using analysis of variance. Individual lambs were treated as replicates, while recognising the lack of independence within groups, because all lambs grazed all replicates in a rotation. Least squares means analysis was used to test the differences between treatments where animal numbers were unbalanced due to deaths. Analysis of variance was used to compare sward surface height; herbage mass, botanical composition, tiller density, nutritive value, alkaloid concentration, using sample dates as replicates.

7.4 RESULTS

The first incidence of ryegrass staggers was at the end of December (Figure 7.5) with three follower lambs showing small degrees of staggering (mean score of 3). On February 10 there was a sharp rise in the incidence and severity of staggers. On February 25 all lambs in all treatments exhibited clinical staggers symptoms. Staggers was more severe than expected and data on performance was based around staggers phases. On 9 March lambs were removed from toxic ryegrass pasture due to the severity of staggers. They were fed lucerne hay and recovered in one week. On 23 March lambs were reintroduced to ryegrass plots but were again removed on 7 April due to a recurrence of severe staggers (mean score of 4.7). Final carcass weights could not be obtained because lambs were unable to be slaughtered due to the severity of their staggers symptoms. The mean daily maximum air temperature (Table 7.1, Appendix 7.3) was higher than the 20-year average at the experimental site for February, March and April. Rainfall was considerably lower than average in November, January and March.

Table 7.1 Weather conditions at the experiment site during summer 1997/1998.

Month	Rainfall (mm)		Mean daily maximum air temperature (°C)	
	1997/98	20-year mean	1997/98	20-year mean
November	57.1	81.9	18.1	18.4
December	103.4	89.9	20.4	20.6
January	31.7	63.3	22.5	22.3
February	61.4	71.3	25.1	22.7
March	35.6	81.3	23.1	20.8
April	72.9	79.8	19.9	18.3

7.4.1 Sward characteristics

Mean pre-grazing sward height was 13.5 cm for the leader lambs and 7.0 cm for the follower lambs (Table 7.2). Mean post-grazing sward height was 7.0 cm for the leaders and 6.0 cm for the followers. Mean pre-grazing herbage mass was 4400 kg DM/ha for the leader lambs and 3800 kg DM/ha for the followers. Mean post-grazing herbage mass was 3800 kg DM/ha for the leader lambs and 3700 kg DM/ha for the follower lambs. Management significantly affected pre-grazing sward height ($P=0.0001$) and herbage mass ($P<0.005$) and post-grazing sward height ($P=0.011$). There were no differences between cultivars in either pre- or post-grazing sward conditions.

Perennial ryegrass was the major contributor to sward composition (Table 7.3), making up over 90% of live herbage in both Aries HD and Yatsyn 1 pastures. The proportion of ryegrass decreased ($P=0.0001$) and dead material increased ($P<0.001$) with grazing. There was no difference between cultivars in ryegrass content, other grasses and dead material.

The proportion of leaf in ryegrass was 70% in both cultivars from January to March (Table 7.4). The proportion of vegetative tillers from mid-December to mid-January (the main period of reproductive development) was higher in Aries HD than Yatsyn 1 pasture but this difference was not statistically significant (Table 7.4 and Figure 7.1). After mid-December over 95% of tillers in both cultivars were vegetative. Ryegrass tiller density was significantly higher in Aries HD than Yatsyn 1 pasture in January (Figure 7.2) but was not statistically different over the entire experiment (Table 7.4). Other grasses made up only a small proportion of the pasture (Table 7.3 and 7.4) and there were no differences between cultivars. Endophyte infection percentage was high in both cultivars and did not change over the experiment (96% in November and April).

Table 7.2 Pre- and post-grazing sward height (cm) and herbage mass (kg DM/ha) under rotational grazing management from 2/12/97 to 10 March 1998 (n=13/trt).

Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Pre-grazing								
Sward height	13.6	13.3	6.9	7.2	1.00	0.9666	0.0001	0.7421
Herbage mass	4550	4300	3740	3880	221	0.7951	0.0072	0.3793
Post-grazing								
Sward height	6.9	7.2	6.0	6.0	0.39	0.5721	0.0106	0.7033
Herbage mass	3740	3880	3720	3690	183	0.7709	0.5778	0.6439

Table 7.3 Relative contribution (% DM) of the components of perennial ryegrass pasture under rotational grazing management from 2/12/97 to 3/3/98 (n=13/trt).

Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Ryegrass	60.1	59.8	48.4	46.4	2.73	0.6798	0.0001	0.7411
Other grasses	4.1	5.0	4.5	4.9	1.25	0.6076	0.9099	0.8323
Dead material	35.8	35.1	47.1	48.7	3.42	0.8870	0.0007	0.7331

Table 7.4 Proportion of leaf in ryegrass (%), proportion of vegetative tillers (%), tiller density (tillers/m²) and the proportion of tillers infected with endophyte (%) in pure swards of Aries HD and Yatsyn 1 perennial ryegrass.

Measurement	Cultivar		SEM	P
	Aries HD	Yatsyn 1		
Proportion of leaf in ryegrass (30/12 to 31/3, n=6/trt)	68.1	69.2	1.646	0.6658
Proportion of vegetative tillers (13/11 to 15/12, n=3/trt)	65.9	55.5	4.70	0.1539
Ryegrass tiller density (2/12 to 31/3, n=9/trt)	4580	3800	328	0.1020
Other grass tiller density (2/12 to 31/3, n=9/trt)	875	956	158	0.7231
Endophyte infection (n=6/trt)				
17/11/97	98.3	93.3	3.03	0.2956
17/4/98	96.7	96.7	1.83	1.0000

Figure 7.1 Proportion of vegetative and reproductive tillers (having a visible seedhead or exhibiting stem elongation) over November to December 1997 in pure swards of Aries HD and Yatsyn 1 perennial ryegrass.

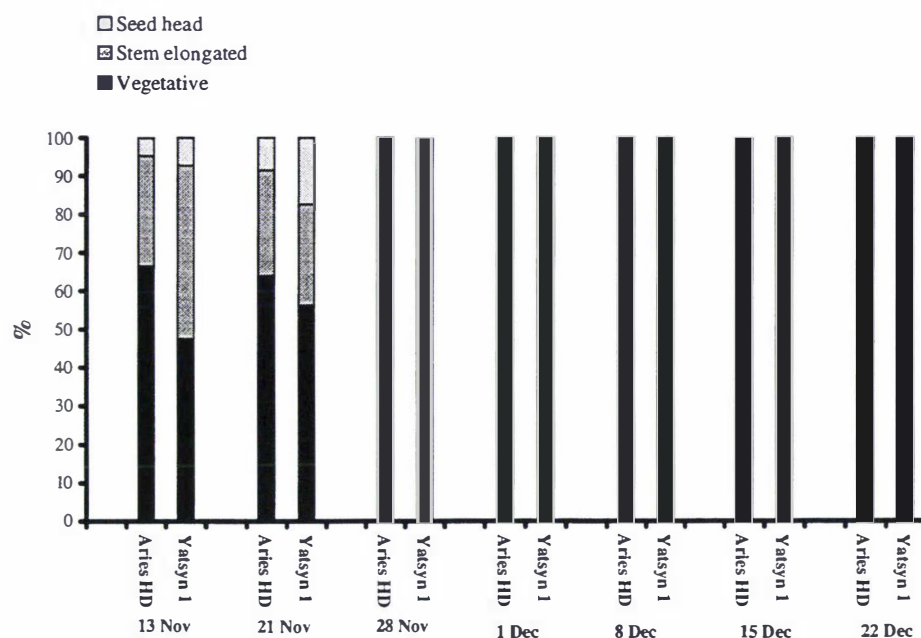
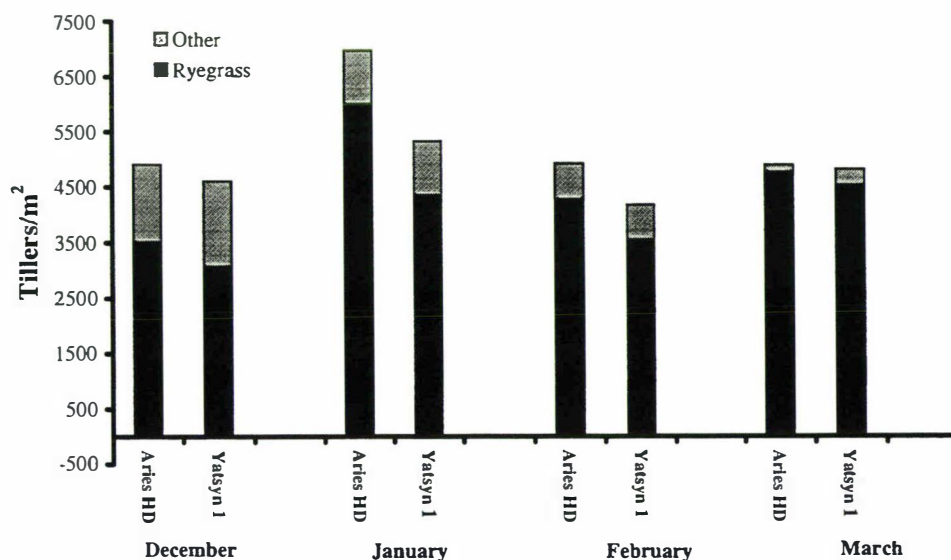


Figure 7.2 Tiller density of ryegrass and other species in pure swards of Aries HD and Yatsyn 1 perennial ryegrass.



7.4.2 Nutritive value

Herbage cut to ground level (herbage on offer) and herbage cut to grazing height did not differ between cultivars in organic matter digestibility, neutral detergent fibre or nitrogen content (Table 7.5, Appendix 7.4 and 7.5). Organic matter digestibility was on average 5 units lower in herbage on offer ($P<0.005$) and herbage selected ($P=0.066$) in the follower swards. Herbage on offer in leader swards was 4% units lower in neutral detergent fibre ($P<0.001$) than in follower swards and there was no significant difference in nitrogen content.

7.4.3 Alkaloid concentration

The maximum concentration of lolitrem B in leader pastures (Figure 7.3 A) was 5.5 ppm on 3 February for Aries HD and 3.9 ppm on 24 February for Yatsyn 1. Maximum concentration in follower pastures occurred on 31 March for Aries HD with 6.0 ppm and on 3 March with 4.1 ppm for Yatsyn 1. Highest ergovaline levels (Figure 7.3 B) on leader pasture occurred on 24 February with 0.21 ppm for Aries HD and 0.72 ppm on 15 April for Yatsyn 1. In the follower pasture highest ergovaline levels were 0.67 ppm in Yatsyn 1 on 10 February and 0.30 ppm in Aries HD on 3 March. Peramine concentration (Figure 7.3 C) increased over the experiment to a peak concentration in leader pastures on 15 April of 19.1 ppm in Aries HD and 22.8 ppm in Yatsyn 1. In follower pastures peramine peaked on 10 February with 14.4 ppm in Aries HD and on 3 March with a concentration of 16.9 ppm in Yatsyn 1 pastures. Cultivar or management did not significantly affect the concentrations of lolitrem B or peramine concentrations in herbage on offer (Table 7.6). However, ergovaline concentrations were significantly affected by cultivar and were two times higher in Yatsyn 1 pastures than Aries HD pastures (0.49 vs 0.21 ppm for Yatsyn 1 and Aries HD respectively, $P=0.0001$). Management did not significantly affect concentration of ergovaline. During April and May concentrations of lolitrem B were 3.0 vs 2.5 ppm, peramine concentrations were 17.8 vs 20.7, and ergovaline concentrations were 0.17 vs 0.59 ppm in Aries HD and Yatsyn 1 pastures, respectively.

Table 7.5 Effect of cultivar and grazing management on *in vitro* organic matter digestibility (OMD%), nitrogen and neutral detergent fibre (%DM) from herbage cut to ground level from 9/12/97 to 31/3/98 (n=5/trt) and OMD from herbage cut to grazing height from 16/12/97 to 7/4/98 (n=5/trt).

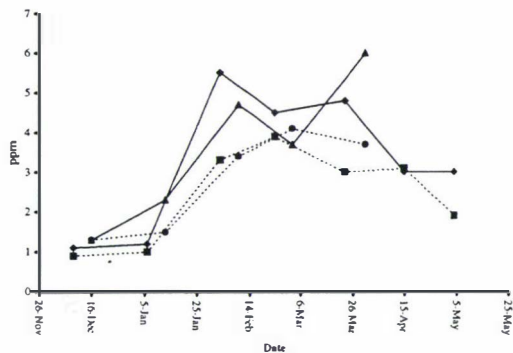
Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Herbage cut to ground level (pre-grazing)								
OMD	60.6	60.7	54.6	55.1	2.37	0.8963	0.0278	0.9252
Neutral detergent fibre	58.9	59.5	63.3	62.9	1.14	0.9273	0.0038	0.6664
Nitrogen	2.06	2.17	1.83	2.00	0.194	0.4888	0.3234	0.8910
Herbage cut to grazing height (after grazing)								
OMD	66.7	64.0	60.3	60.1	2.62	0.6020	0.0660	0.6349

Table 7.6 Effect of cultivar and grazing management on endophyte alkaloid estimates (ppm) from herbage cut to ground level from 9/12/97 to 31/3/98 (n=5/trt).

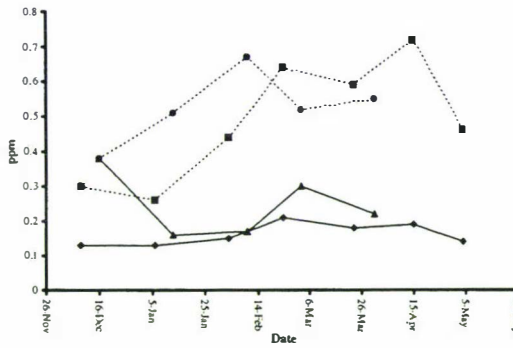
Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Lolitre B	3.42	2.42	3.60	2.80	0.769	0.2531	0.7171	0.8968
Peramine	11.12	14.4	9.94	12.76	1.790	0.1077	0.4423	0.8993
Ergovaline	0.16	0.45	0.25	0.53	0.050	0.0001	0.1136	0.9525

Figure 7.3 Alkaloid concentrations in herbage cut to ground level in Aries HD leader (◆), Yatsyn 1 leader (■), Aries HD follower (▲) and Yatsyn 1 follower (●) pastures over the experiment: **A**, Lolitrem B; **B**, Ergovaline; **C**, Peramine.

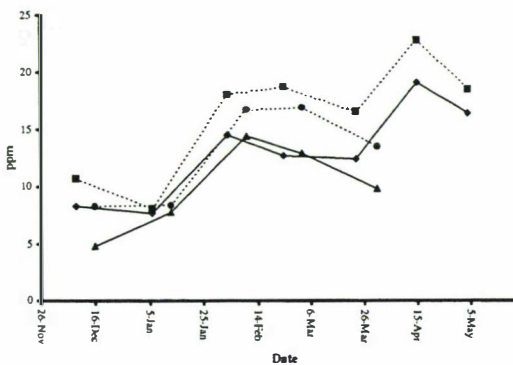
A



B



C



7.4.4 Herbage intake and liveweight gain

The release rate of chromium estimated from capsules collected from experimental lambs in the previous experiment (Chapter 5) did not differ between lambs grazing Aries HD and Yatsyn 1 pastures, so a common release rate of chromium (140.6 ± 2.67 mg/day) was used to determine faecal output. Herbage intake of lambs (Table 7.7) was significantly higher on Aries HD pasture than Yatsyn 1 pasture (0.95 vs 0.85 kg OM/day, $P < 0.005$). There was no significant effect of management on herbage intake, and no difference in organic matter digestibility over the intake period from herbage sampled by oesophageal fistulated sheep between cultivars or between leader and followers. Organic matter digestibility for follower pasture was high over the intake period (mean value of 81%).

There was significantly faster liveweight gain in leader lambs than in follower lambs (Table 7.8) resulting in a highly significant effect of management on liveweight from January 6 onwards (Figure 7.4). Over the entire experiment leader lambs grew at 92 g/day compared to only 53 g/day for follower lambs ($P < 0.001$). However, there was no significant difference in lamb liveweight gain between cultivars either before severe staggers was observed or during severe staggers.

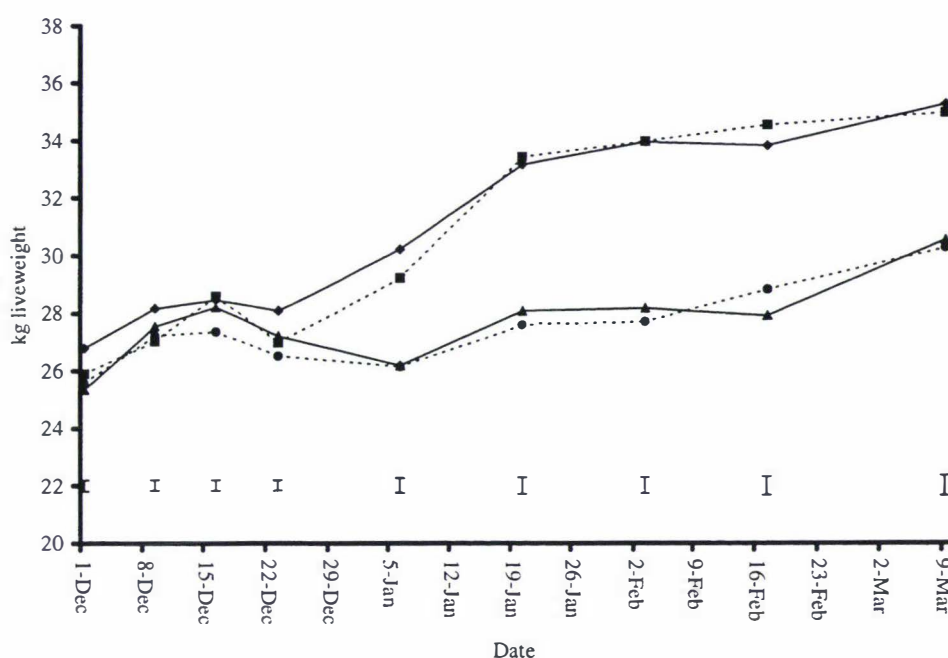
Table 7.7 Effect of cultivar and grazing management on herbage intake and organic matter digestibility from oesophageal fistulate samples (n=5/trt) from 20/1/98 to 29/1/198.

Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Organic matter digestibility (%)								
Week 1	82.5	80.6	83.4	83.6	1.93	0.6606	0.3200	0.5921
Week 2	76.5	75.2	79.5	77.4	2.67	0.5749	0.3859	0.8751
Herbage intake (kg OM/day)								
No of animals	15	15	14	14	-	-	-	-
Week 1	1.14	1.03	1.08	1.00	0.042	0.0212	0.3258	0.6562
Week 2	0.84	0.66	0.74	0.70	0.033	0.0011	0.3853	0.0298
Overall	0.99	0.84	0.91	0.85	0.023	0.0018	0.2831	0.1581

Table 7.8 Effect of cultivar and grazing management on initial and final lamb liveweight (kg). Liveweight gain (g/day) before severe ryegrass staggers was observed, during severe staggers and over the entire experiment.

Lamb performance	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Liveweight (kg)								
Initial no of animals	15	15	15	15	-	-	-	-
Initial (1/12)	26.8	25.9	25.4	25.6	0.56	0.5237	0.1426	0.3445
Final no of animals	15	12	10	11	-	-	-	-
Final (9/3)	35.3	35.0	30.6	30.3	1.19	0.7930	0.0001	0.9879
Liveweight gain (g/day)								
Before severe staggers								
(1/12 to 3/2: 64 days)	112	126	44	33	10.4	0.8508	0.0001	0.2267
During severe staggers								
(3/2 to 9/3: 34 days)	39	42	83	81	25.3	0.9758	0.0876	0.9216
Overall								
(1/12 to 9/3: 98 days)	87	96	55	50	10.6	0.7350	0.0003	0.4844

Figure 7.4 Effect of cultivar and grazing management on liveweight over time. Aries HD leaders (◆); Yatsyn 1 leaders (■); Aries HD followers (▲); Yatsyn 1 followers (●). Bars indicate SED 5%.



7.4.5 Incidence and severity of staggers and heat stress

Both cultivar and management (Table 7.9) significantly affected staggers score over the severe staggers period from 10 February to 9 March ($P < 0.01$ for cultivar and $P = 0.005$ for management). Staggers score was highest in the Yatsyn follower group and lowest in the Aries HD leader group (Table 7.9). Cultivar and grazing management also significantly affected respiration rate. Yatsyn 1 leader lambs had the highest respiration rate of 78.3 breaths per minute versus 73.3 breaths per minute in the Aries HD follower lambs ($P = 0.060$ for management and $P = 0.001$ for cultivar). Management significantly affected serum prolactin concentration with Yatsyn 1 follower lambs having the lowest concentration in their blood ($P < 0.005$). Rectal temperature was not significantly affected by either cultivar or grazing management. Faecal moisture measured on two occasions was the same between treatment groups and was 10% higher in February than in

January. Faecal dag score was significantly lower in the follower lambs than in the leader lambs ($P < 0.05$) and was unaffected by cultivar.

Figure 7.5 Effect of cultivar and management on severity of ryegrass staggers. Bars indicate SED 5%.

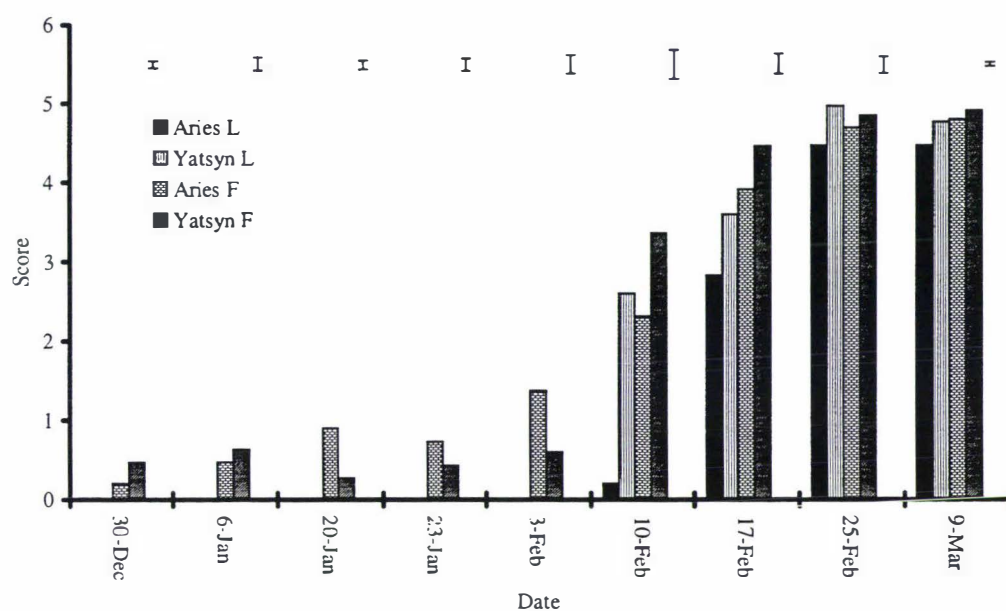
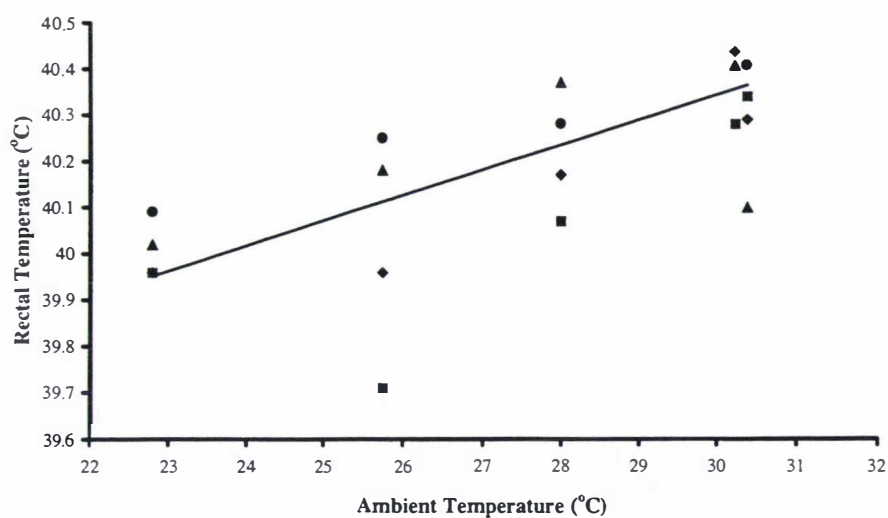


Table 7.9 Effect of cultivar and grazing management on several indicators of animal health for rotationally grazed lambs.

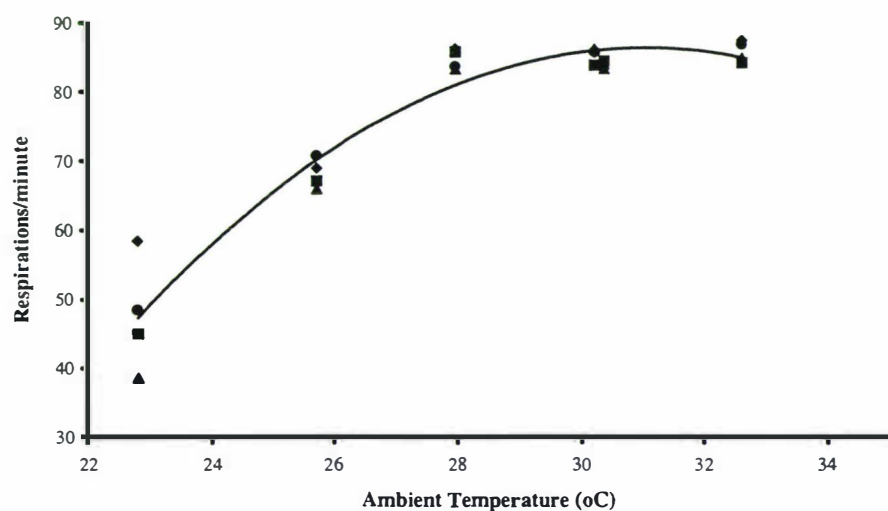
Measurement	Leaders		Followers		SEM	Statistical Significance (P-value)		
	Aries HD	Yatsyn 1	Aries HD	Yatsyn 1		Cultivar	Management	Interaction
Staggers score (0-5)								
10/2/97 to 9/3/98	3.1	3.9	4.0	4.4	0.23	0.0091	0.0034	0.3797
Rectal temp. (°C)								
16/1/98 to 17/2/98	40.1	40.1	40.1	40.3	0.07	0.6435	0.1497	0.0733
Respiration rate (breaths/minute)								
16/1/98 to 17/2/98	75.0	78.3	73.3	76.5	1.13	0.0010	0.0603	0.9453
Serum prolactin cc. (ng/ml)								
3/2/98 to 13/2/98	199.0	163.6	126.0	113.8	18.04	0.1811	0.0012	0.5185
Faecal moisture (%)								
30/1/98 and 13/2/98	75.0	73.5	74.0	75.8	1.71	0.9756	0.7780	0.4977
Faecal dag Score (0-5)								
6/1/98 to 9/3/98	1.8	1.6	1.1	1.0	0.33	0.6946	0.0341	0.8491

Figure 7.6 Effect of increasing ambient temperature on: A, Rectal temperature and B, Respiration rate of lambs. Aries HD leaders (◆); Yatsyn 1 leaders (■); Aries HD followers (▲); Yatsyn 1 followers (●); Mean of 4 treatments (—).

A



B



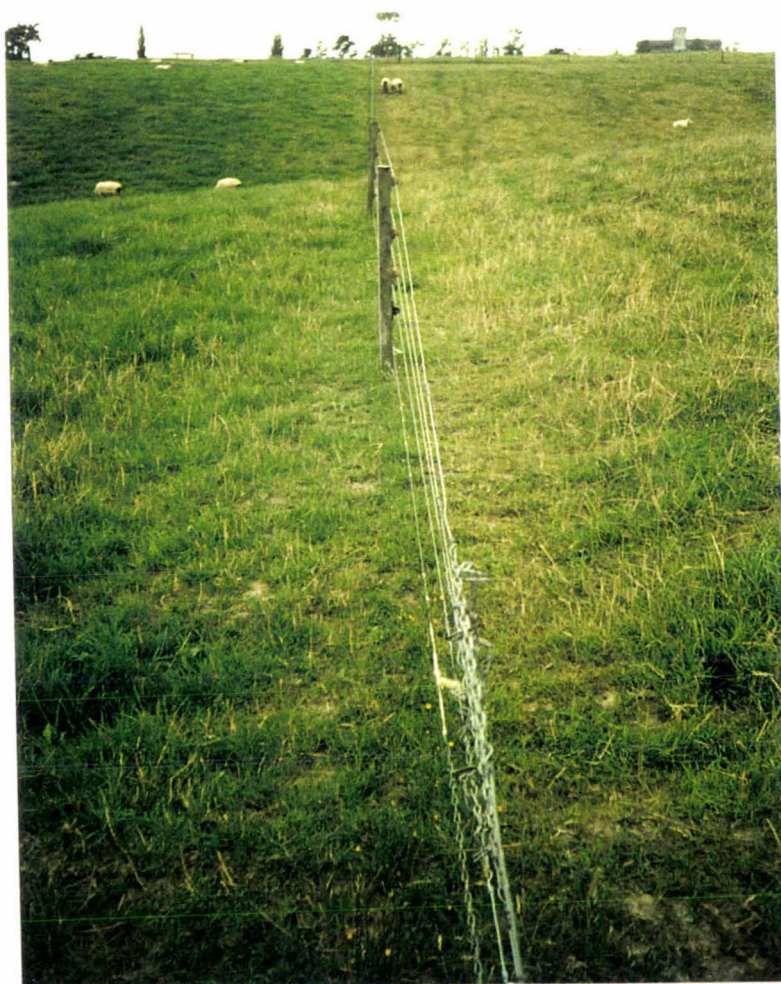
It was observed that body temperature and respiration rate increased as ambient temperature increased (Figure 7.6), although ambient temperature was not an imposed treatment. Heat stress measurements were made covering an ambient temperature range from 23 °C to 33 °C. A significant linear relationship ($R^2=0.7972$) was found between ambient temperature (x) and the rectal temperature of lambs (Figure 7.6 A) with the equation being $0.0542x + 38.718$. A significant quadratic relationship ($R^2=0.9786$) was found between ambient temperature (x) and respiration rate (Figure 7.6 B) with the equation being $-0.5667x^2 + 35.23x - 461.27$.

7.5 DISCUSSION

The leader-follower grazing management created differences in sward height, herbage mass and botanical composition (Plate 7.3). Liu et al. (1998) has demonstrated similar contrasts resulting from a leader-follower grazing sequence by lambs grazing Yorkshire fog pastures. There was a considerable reduction in herbage mass and proportion of ryegrass on offer after grazing by the leader groups, and this decreased the opportunity for selective grazing by follower lambs. The contrast in pre-grazing herbage mass and sward height declined with each successive rotation compared with the contrast when the experiment began in December.

It was anticipated that the leader lambs would test the nutritional value of the pasture by being offered leafy pasture after a regrowth period of 4 weeks. Wrightson Seeds Ltd have demonstrated higher levels of organic matter digestibility in Aries HD than in Yatsyn 1 pastures (87.2 vs 84.6%) cut to grazing height every 4 weeks and allowed to regrow (Appendix 3.2). Our management simulated these conditions, but no contrast in digestibility was detected from herbage cut to ground level or to grazing height, or from oesophageal fistulate samples. Furthermore, no differences in herbage organic matter digestibility were detected under continuous grazing management in previous experiments in this series (Chapter 3, 4 and 5).

Plate 7.3 Contrasts in sward conditions between leader pre-grazing pasture on the left and follower pre-grazing pasture on the right in Experiment 4.



Grazing height has a major effect on the development and severity of ryegrass staggers. Low grazing height ensures maximum intake of potentially toxic ryegrass tissue (Fletcher and Piper 1990). Follower swards contained 10% more dead material than leader swards. Increased tremorgens will be acquired as the proportions of dead ryegrass material increase in the diet (Fletcher and Piper 1990). However, the concentration of lolitrem B was not significantly higher in the follower pasture (Table 7.6). It is possible that other tremorgens such as paxilline may have contributed to the incidence of staggers (Fletcher et al. 1993). The first signs of staggers emerged in the follower groups on 30 December. Three lambs exhibited mild staggering over January and were lambs probably more susceptible to the condition (Fletcher et al. 1990).

On February 10 there was a sharp rise in the incidence and severity of staggers, coinciding with an increase in lolitrem B concentration. A lamb with staggers is shown in Plate 7.4. On farms in Palmerston North, there were other reports of severe outbreaks of ryegrass staggers in February through to March, coinciding with high ambient temperatures and low rainfall. Lolitrem B levels remained above the 2 ppm threshold believed to induce clinical staggers (di Menna et al. 1992; Blythe et al. 1993) for the majority of the experiment. Pastures contained no companion white clover to dilute the intake of toxins (Fletcher 1993a). Both cultivar and management significantly affected severity of staggers observed. Eerens et al. (1998) reported that follower lambs suffered more severely from staggers, with an average score of 0.65 versus 2.27 for leaders and followers respectively during March. Pownall et al. (1993) has also reported increased severity of staggers as the proportion of pseudostem in the diet increased.

Plate 7.4 Lamb with ryegrass staggers during Experiment 4.



Plate 7.5 Lambs showing signs of heat stress in Experiment 4.



Yatsyn 1 lambs were significantly more seriously affected by staggers than Aries HD lambs. Lolitrem B concentration did not differ significantly between cultivars. However, ergovaline concentration in Aries HD was consistently half those of Yatsyn 1 samples. Tremorgenic alkaloids may act synergistically with other endophyte/ryegrass alkaloids in the incidence of staggers (Gallagher et al. 1977; Fletcher et al. 1993a; Clarke et al. 1996). This potential for increased toxicity through possible synergy has been reported in an experiment where lambs grazing ryegrass pastures with 50 % wild-type endophyte and 50 % AR6 endophyte (no lolitrem/high ergovaline) had similar levels of staggers to those grazing 100% ryegrass with wild-type endophyte, despite a 50% reduction in the concentration of lolitrem B (Fletcher and Easton 1997). In Chapter 3 it was reported that Aries HD herbage had similar lolitrem B concentrations but significantly lower ergovaline levels than in Yatsyn 1 herbage, and a lower incidence of ryegrass staggers in lambs grazing Aries HD pasture. Reduced liveweight gain in Yatsyn 1 pastures was probably related to differences in ergovaline concentrations and staggers occurrence.

Serum prolactin is very sensitive to low levels of ergovaline and can be a good indicator of intoxication with ergopeptine alkaloids such as ergovaline (Fletcher and Easton 1997). Prolactin concentration was significantly reduced in follower lambs. Ergovaline concentrations are highest in the basal vegetative tissue that if grazed may pose a risk to animal health (Lane et al. 1997). The concentration of ergovaline was 28% higher in follower swards than in leader swards, but this difference was not significant. Ergovaline levels of 0.5 to 1.0 ppm are in a comparable range to those associated with symptoms of toxicity in endophyte-infected tall fescue in south east USA (Easton et al. 1993). Feeding endophyte-infected tall fescue seed to lambs with ergovaline levels 0.5 to 2.0 significantly reduced feed intake and average daily gain as compared to controls (Debassai 1993). Significant prolactin responses have been recorded in lambs grazing pastures with ergovaline concentrations of 0.5 ppm (Fletcher et al. 1994). Ergovaline concentrations exceeded 0.5 ppm in Yatsyn 1 pastures over mid-January to April.

Both management and cultivar significantly affected respiration rate of lambs over a range of 23 to 33 °C, but there were no significant differences in rectal temperatures

between treatments. Respiration rate is thought to be a more sensitive measure of heat stress than rectal temperature (Fletcher 1993b). It can not be concluded whether respiration rate and serum prolactin levels observed were different to normal sheep because an endophyte-free control was not included in the experiment to establish base levels (Fletcher and Easton 1997). Lambs showing signs of heat stress are shown in Plate 7.5. However, it is apparent that differences exist between different managements and cultivar/endophyte associations. Although ambient temperature was not an imposed treatment in this experiment, rectal temperature and respiration rate increased as ambient temperature increased, an observation which has been reported elsewhere (Fletcher 1993b; Fletcher et al. 1994).

Reduced herbage intake is a normal thermo-regulatory response to hyperthermia (Fletcher and Easton 1997). The higher concentration of ergovaline in Yatsyn 1 pasture could explain the lower herbage intake of lambs grazing Yatsyn 1 pastures during late January. Organic matter digestibility did not differ between treatments measured using oesophageal fistulate samples. Actual estimates for herbage intake were similar to predicted values based on energy requirements (1.2 vs 0.9 kg OM/day for leaders and followers respectively; Geenty and Rattray 1987). However, there was no significant difference between the intakes of leader and follower lambs which was unexpected due to large differences in botanical composition and nutritive value between pastures. Liveweight gain was extremely low in all treatments over the intake measurement period possibly due to the constant handling impairing performance (40 vs 6 g/day for leaders and followers, respectively). The severe and debilitating symptoms of ryegrass staggers could have been obscuring the effects of differences in ergovaline concentration and heat stress between cultivars and any effects on resulting animal performance during the period when severe staggers were observed. Average staggers severity score was greater than 3.0 which is characterised by marked trembling of general musculature and head shaking; some lack of co-ordination of movement and impaired vision while running (Keogh 1973).

Faecal dag score was significantly greater in leader lambs, though this difference was not observed in faecal moisture percentage. Pownall et al. (1993) also reported greater

faecal soiling in lambs being offered higher pasture allowances, ranging from a faecal soiling score of 2.5 in lambs having first offer to 1.1 in those grazing the base of the plants. Lambs grazing endophyte-infected ryegrass often have increased faecal scouring (0.2 vs 2.5 dag score; Fletcher et al. 1996). Paxilline or other unidentified alkaloids may be involved, but it has not been possible to make a definite link between faecal contamination and specific toxin(s) (Pownall 1995; Fletcher 1993a).

Liveweight change can reflect the dominant effects of management, ryegrass cultivar or endophyte effects which encompasses many interrelated and significant primary effects including alkaloid concentrations, ryegrass staggers, heat stress and faecal contamination. Over the entire experiment leader lambs gained 92 g/day compared to only 53 g/day for lambs on follower treatments. Despite the relatively high herbage mass on offer in the follower pastures, lambs were forced to consume more stem and dead material left behind by the leader lambs. This resulted in the reduced liveweight gains observed. Pownall et al. (1993) grazed endophyte-infected hybrid ryegrass successively by four groups of lambs using a leader-follower system and liveweight gain ranged from 164 g/day in lambs having first offer, to 28 g/day in those grazing the base of the plants. Liveweight gain was reduced in the leader lambs by 100% during severe staggers, while follower lambs showed unexpected increases in liveweight gain. Differences in ergovaline concentrations, heat stress symptoms and ryegrass staggers severity did not result in any significant difference in liveweight gain overall between cultivars in this experiment.

7.6 CONCLUSIONS

The occurrence of staggers was extreme in the current study but was more severe in Yatsyn 1 pastures than in Aries HD pastures. Higher ergovaline concentrations may have acted synergistically with lolitrem B concentrations to increase the severity of staggers observed in lambs grazing Yatsyn 1 pastures. Respiration rate, which is an indicator of heat stress, was higher in Yatsyn 1 lambs.

The contrast in sward conditions in leader and follower swards resulted in substantial reductions in organic matter digestibility and consequent liveweight gain of follower lambs. Follower lambs had significantly reduced serum prolactin levels and respiration rates, possibly reflecting greater ergovaline intoxication.

The results of this study and previous studies in the series show that grazing management and alkaloid challenge can determine the performance of weaned lambs grazing ryegrass over summer and autumn. The effect of management and alkaloid concentration is likely to have a larger impact on lamb performance than small differences in nutritive value between cultivars of high-endophyte perennial ryegrass. There is also some indication that heat stress symptoms are more severe in lambs forced to graze lower into the sward.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1 INTRODUCTION

Feeding value is the biological worth of a forage in terms of animal production (Ulyatt 1973). Gains from improved digestibility must be validated in animal experiments (Vogel and Sleper 1994), but the complexity of the interactions between factors contributing to feeding value can make it extremely difficult to identify specific reasons for differences in performance in a given situation (Black 1990). Endophyte (*Neotyphodium lolii*) also has an impact in cultivar evaluations, as the presence of endophytes can increase biotic and abiotic host tolerance and cause toxicosis in grazing animals due to alkaloids (Ravel et al. 1997). Liveweight gain differences between perennial ryegrass cultivars can be related to differences in nutritive value, contrasts in cultivar/endophyte, or a combination of both.

The initial objective in this thesis was to make comparative evaluations of Aries HD selected for improved digestibility, with a control cultivar Yatsyn 1 perennial ryegrass, with reference to performance of lambs, herbage intake, nutritive value and grazing behaviour. After the first weaned lamb experiment over summer and autumn (Chapter 1) it was apparent that the cultivars differed in the occurrence and severity of staggers and in their concentration of endophyte alkaloids. A second aim was then incorporated to evaluate the effects of the respective cultivar/endophyte associations on the production of alkaloids, and on the health and performance of sheep.

The results of individual experiments have been discussed in detail in the preceding Chapters. Following is an evaluation of the experimental procedures used in the grazing studies. This Chapter will then focus general discussion on 1) the comparative feeding

value of Aries HD and Yatsyn 1 perennial ryegrass; 2) effects due to improved nutritive value and digestibility; 3) effects due to the perennial ryegrass/endophyte association; 4) the implications for plant breeding, Aries HD and endophytes in the future.

8.2 EVALUATION OF THE EXPERIMENTAL PROCEDURES USED IN THE CURRENT RESEARCH PROGRAMME

8.2.1 Measurement of animal performance and herbage intake

One of the main objectives of this study was to compare the feeding value or animal production response of Aries HD and Yatsyn 1. Animal production was assessed in four grazing experiments where intake was not limiting. This was to ensure that contrasts in animal performance were due to nutritional quality only and not a difference in the quantity of herbage eaten. Animals were allocated to treatments by stratified randomisation to take into account liveweight and sex. The coefficient of variation ranged between 11 and 14% for liveweight gain and 27 to 28% for carcass weight gain, reflecting the large variation between animals. The coefficient of variation for clean wool production ranged between 16 to 19%. A minimum response period of 30 days grazing is needed to accurately assess treatment affects on liveweight gain (Fletcher and Easton 1997). The duration of all the grazing experiments in this study were in excess of three months. Intensive handling periods such as the measurement of herbage intake, and symptoms of staggers and heat stress would have adversely affected animal performance (Corbett 1978) and increased variability.

The values of herbage intake reported in this study were estimated from faecal output and diet digestibility. Faecal output of the experimental lambs was estimated indirectly using intra-ruminal chromium (Cr_2O_3) controlled release capsules in the procedure described by Parker et al. (1989; 1990). The digestibility of the diet was based on herbage samples collected with oesophageal fistulated sheep. Intake estimates were low compared to predictions made based on energy requirements in the lactation experiment (Chapter 4: 1.55 vs 2.4 kg OM/day) and in the subsequent lamb finishing experiment (Chapter 5: 0.61 vs 1.4 kg OM/day). It is possible that *in vitro* organic matter

digestibility was underestimated, since at high levels of digestibility small errors in estimates can exert a disproportionate influence on calculation of herbage intake (Parker et al. 1990). Organic matter digestibility values estimated from hand plucked samples during the intake periods were on average 2 to 5% lower than from samples selected by oesophageal fistulated sheep, so were not used to estimate herbage intake.

Errors in estimates of herbage intake can also arise from differences between capsules in marker release rate and between-animal variations in the digestibility of the diet selected by oesophageal fistulated sheep (Parker et al. 1990). The coefficients of variation in organic matter digestibility of oesophageal fistulate samples were relatively small, between 3 and 8%. The release rate of chromium may be dependent on the particular forage being tested (Burns et al. 1994). This was shown in Chapter 3 with a significant difference in chromium release rate between Aries HD and Yatsyn 1 pasture (165 vs 155 mg/day, $P < 0.001$). However, in the following summer experiment release rates were similar between cultivars (139 vs 142 mg/day, $P = 0.4754$). There are often large deviations between manufacturer-specified release rates (133 mg/day) and observed release rates (Burns et al. 1994). Chromium release rates derived from capsules in rumen fistulated sheep during Experiment 1 were too low and variable to be used (110 mg/day). The choice of release rate may have introduced minor errors in estimating intake, but not enough to explain the large difference between calculated herbage intake and predicted intake based on energy requirements. The coefficients of variation in herbage intake were between 14 and 28% in the current study, and are similar to others reported at Massey University (13 – 24%; Liu 1996). Burns et al. (1994) warned that the use of slow release devices must proceed with caution.

8.2.2 Measurement of staggers and heat stress symptoms

Ideal conditions for the development of staggers have been reviewed in Chapter 2.4. Animals should graze ryegrass/endophyte associations for a minimum of three weeks at times when alkaloid challenge is highest (Fletcher and Easton 1997). Procedures for evaluating heat stress symptoms such as rectal temperatures and respiration rates are still being developed. Measurement of these variables requires stable environmental

conditions and ambient temperatures over 22 °C (Fletcher and Easton 1997). On average the maximum monthly air temperature is greater than 22 °C in January and February in the Manawatu (Appendix 8.1). Experiment 4 (Chapter 7) lambs were kept inside a closed shed to keep the air temperature both constant and high. The coefficient of variation for respiration rate was 10%, and for rectal temperature 1%.

Serum prolactin can be a good indicator of intoxication with ergopeptine alkaloids such as ergovaline (Fletcher and Easton 1997). The coefficient of variation for serum prolactin concentration was high, between 54 and 63%, which is a characteristic of this measurement. For this reason blood sampling was repeated over successive days during similar environmental conditions. A contrast in prolactin concentration was apparent between Aries HD and Yatsyn 1 in Experiment 4 (Table 7.9) but due to the inherent variability in this measurement this difference was not significant. Fletcher et al. (1997) has reported that black-globe temperature, which also takes into account solar radiation, has a higher correlation with prolactin concentration and respiration rate than does ambient temperature. An endophyte-free control should be included in evaluations of ryegrass/endophyte associations to obtain a 'normal' level of rectal temperature, respiration rate and serum prolactin concentration (Fletcher and Easton 1997). However, including endophyte-free treatments was not feasible in the current research programme.

8.2.3 The use of pure swards

Cultivars were evaluated in pure swards to avoid the complications of varying percentages of clover between plots. However, *Poa annua* was a major (but similar) weed contaminant in both cultivars in Experiments 2 and 3 (43% of live herbage). Bircham and Hodgson (1982) found that in continuously stocked swards, *Poa annua* tended to be grazing-evasive, retaining leaf area and mass because its leaves were less accessible to defoliation than those of its competitors. Therefore, *Poa annua* was probably not a major contributor to the diet, but may have diluted contrasts between cultivars. It was therefore decided to eliminate *Poa annua* for the final grazing experiment by applying Nortron herbicide (Ethofumesate; BASF, NZ Ltd.). This

method was successful with the weed grass component being less than 5 % in live herbage (Table 7.3).

Differences in animal performance obtained in pure swards may not occur in mixed swards (Snaydon 1979). Cultivars should also be evaluated in mixtures to investigate compatibility with legumes. Variability between cultivars in seasonal growth patterns and/or morphology can influence the growth of companion white clover (Davies et al. 1993; Reed 1994; Elgersma and Schleepers 1997). Differences in animal performance between cultivars of perennial ryegrass have been shown to be more pronounced as mixed swards with white clover (Munro et al. 1992; Davies et al. 1993). Endophyte in grasses may also influence the presence and production of companion legumes in mixed pastures, especially under seasonally dry conditions (Fletcher and Easton 1997). There is a need to evaluate new cultivars bred for increased nutritive value as pure swards to evaluate whether the breeding programme has been successful, and as mixed swards with companion clover to assess compatibility.

8.2.4 Replication

In planning an experiment a balance has to be achieved between the number of replicate plots per treatment and the number of samples per plot. The coefficient of variation must be kept as small as possible (Thomas and Laidlaw 1993). The first three grazing experiments were designed as a randomised complete block with three replicates (experimental units) of 0.33 ha in size. Plots could carry eight weaned lambs over summer or six ewes with suckling single lambs. Increasing the number of replicates to four would have increased the precision and ability to detect significant differences between the two cultivars. However, there was a fixed research area, which would have meant smaller sized plots, smaller number of animals per plot and possibly biased results from inadequate sampling. In the final grazing experiment plot replicates were increased to six. Individual lambs were treated as replicates, while recognising the lack of independence within groups, because groups of lambs grazed all replicate blocks in rotation. Davies et al. (1991) has clearly shown significant trends in animal production between cultivars of perennial ryegrass over three years despite contrasting climatic

conditions. The pooled analysis of liveweight gain and herbage intake over the four experiments (Table 8.1 and 8.2) provides a good indication of general lamb performance over four seasons, at two experimental sites and under both continuous and rotational grazing management.

8.3 COMPARATIVE FEEDING VALUE OF ARIES HD AND YATSYN 1 PERENNIAL RYEGRASS

Experiment 1 (Chapter 3) demonstrated that lambs grazing Aries HD achieved higher liveweight gains than lambs grazing Yatsyn 1 perennial ryegrass. Liveweight gain was significantly higher during summer dry conditions (Table 3.1) and overall (Table 8.1). This resulted in greater carcass weight at slaughter for Aries HD lambs over Yatsyn 1 lambs (16.8 vs 15.4 kg). The better performance achieved on Aries HD pasture than on Yatsyn 1 pasture could not be attributed to any difference in herbage nutritive value (Table 3.4) or herbage intake (Table 3.3). The results suggested that contrasts in lamb performance may have reflected differing alkaloid concentrations from the respective ryegrass/endophyte associations, and consequent effects of staggers. On average 15% of lambs grazing Aries HD pasture were severely affected by staggers compared with 29% of lambs on Yatsyn 1 pastures (Table 3.3). Although the lolitrem B concentrations were similar between cultivars, ergovaline content in Aries HD herbage samples was consistently half that of Yatsyn 1 samples (Table 3.5). These results highlighted the need to investigate the effects of endophyte/cultivar interactions in future evaluations.

Experiment 2 (Chapter 4) measured performance from ewes with suckling single lambs. This enabled a comparison using animals under a high physiological demand for nutrients in the spring, before the effects of endophyte alkaloids are apparent (di Menna 1992; Fletcher et al. 1996). Over September ewes grazing Aries HD gained 40 g/day more than ewes grazing Yatsyn 1 pasture (Table 4.5). This enabled Aries HD ewes to gain an extra kilogram over the spring months (4.4 vs 3.3 kg total liveweight gain for Aries HD and Yatsyn 1 respectively). Suckling lamb growth rates were not different between cultivars, presumably reflecting high and non-limiting milk yields on both cultivars. The better performance of ewes on Aries HD was attributed to higher herbage

intake (Table 4.4). Percentage of ryegrass leaf was higher on Aries HD than on Yatsyn 1 pasture (Table 4.1), but there was again no difference in herbage nutritive value between cultivars (Table 4.2). It is possible that the contrast in leaf content may have been too small to be reflected in a significant difference in nutritive value. It is doubtful that the better performance of ewes grazing Aries HD was entirely due to the higher leaf content of the sward observed in this experiment.

Experiment 3 compared the performance of weaned lambs over the following summer-autumn period. Liveweight gain, carcass weight gain and wool production were similar between lambs grazing Aries HD and Yatsyn 1 ryegrass (Table 5.6). There was also no significant difference in herbage intake, grazing behaviour, or nutritive value of herbage between cultivars (Table 5.3 and 5.5). The incidence of staggers was low and no difference was detected in lamb rectal temperature and serum prolactin concentration (Table 5.7), reflecting low concentrations of endophyte alkaloids (Table 5.4). These results demonstrated no difference in feeding value between cultivars over this particular summer/autumn period.

Results to date indicated that the production response of lambs over summer and autumn reflected the level of alkaloid challenge in the respective pastures. Under continuous stocking management there were no differences in the *in vitro* organic matter digestibility, nitrogen or neutral detergent fibre content from herbage samples cut to ground level, from hand plucked samples or from oesophageal fistulate samples. Therefore a new experiment was instigated (Experiment 4) using rotational grazing to better simulate conditions under which improvement in digestibility had been demonstrated.

Lambs were rotationally grazed in a leader-follower sequence on each cultivar to assess both the nutritive value of the pastures and the alkaloid challenge imposed by the respective endophyte/cultivar associations (2 x 2 factorial design). The leader-follower regime created differences in pre-grazing sward height, herbage mass and botanical composition (Table 7.2 and 7.3). Leader pastures contained a higher proportion of ryegrass and were higher in *in vitro* organic matter digestibility and neutral detergent

fibre (Table 7.5). Leader lambs had significantly faster liveweight gains (Table 7.8) and were 5 kg heavier than follower lambs in March. However, cultivars did not differ in organic matter digestibility or in lamb liveweight gain. Staggers was most severe on Yatsyn 1 follower pastures (Table 7.8). Respiration rate, an indicator of heat stress was significantly higher on Yatsyn 1 pasture, corresponding to consistently higher ergovaline concentration in herbage samples from Yatsyn 1 pasture (Table 7.6). Herbage intake was significantly better in lambs grazing Aries HD than Yatsyn 1 pasture (Table 7.7). It is suggested that the severity of staggers symptoms obscured differences in ergovaline concentration and heat stress between cultivars and any resulting effects on animal performance during the period when severe staggers was observed.

A summary of liveweight gain and herbage intake from the four experiments is given in Tables 8.1 and 8.2. Herbage intake when pooled across the four evaluations was significantly greater in Aries HD lambs than in Yatsyn 1 lambs (1.14 vs 0.96 ± 0.042 kg OM/day, $P = 0.0581$). Mean liveweight gain did not differ significantly between Aries HD and Yatsyn 1 when pooled across experiments (94 vs 86 ± 4.6 g/day, $P = 0.3057$). The feeding value of these two cultivars was the same in all comparisons at the 10 % level of probability as determined by liveweight gain. However, had there been more replicates in Experiments 1 and 2, there is a good indication that animal performance would have been superior at a 5 % level of probability on Aries HD pasture.

Table 8.1 Summary of liveweight gain from four grazing experiments on Aries HD and Yatsyn 1 pastures.

Experiment	Animals	Duration	Grazing Management	Liveweight gain		SEM	P
				(g/day)			
				Aries HD	Yatsyn 1		
1	Weaned lambs	11/12/95 to 25/4/96	Continuous	104	84	4.6	0.1028
2	Ewes	3/9/96 to 1/12/96	Continuous	69	53	9.3	0.1203
3	Weaned lambs	2/12/96 to 12/3/97	Continuous	116	111	5.1	0.6542
4	Weaned lambs ¹	2/12/97 to 23/3/98	Rotational	87	96	10.6	0.7350
Pooled mean				94	86	4.6	0.3057

¹ Figures are for leader lambs only

Table 8.2 Summary of herbage intake from four grazing experiments on Aries HD and Yatsyn 1 pastures.

Experiment	Animals	Duration	Grazing Management	Herbage intake (kg OM/day)		SEM	P
				Aries HD	Yatsyn 1		
1	Weaned lambs	11/12/95 to 25/4/96	Continuous	1.18	1.05	0.046	0.1624
2	Ewes	3/9/96 to 1/12/96	Continuous	1.72	1.37	0.038	0.0322
3	Weaned lambs	2/12/96 to 12/3/97	Continuous	0.65	0.57	0.095	0.5715
4	Weaned lambs ¹	2/12/97 to 23/3/98	Rotational	0.99	0.84	0.023	0.0018
Pooled mean				1.14	0.96	0.042	0.0581

¹ Figures are for leader lambs only

8.4 EFFECTS DUE TO NUTRITIVE VALUE AND DIGESTIBILITY

Animal scientists and plant breeders have ranked increasing organic matter digestibility as the most important goal for improving milk production, liveweight gain and wool production (Wheeler and Corbett 1979; Smith et al. 1997). Wrightson Seeds specifically selected Aries HD for improved whole plant organic matter digestibility. Organic matter digestibility is the only criterion that integrates all components of digestibility, including leaf to stem ratio and the proportion of cell and structural components in the plant. If there is genetic variation for any one of these components then whole plant digestibility is likely to be improved (Vogel and Sleper 1994). Significant genetic variation or heritability has been reported for organic matter digestibility in perennial ryegrass in the literature (Hacker 1982; Dennis and Frandsen 1986). Some advance can still be expected in a species already high in digestibility such as perennial ryegrass, particularly at advanced maturity or seedhead stage (Cooper et al. 1962), when digestibility is inherently at a low level. However, variation in digestibility of plants over maturity may be due to a difference in earliness of flowering (Walters et al. 1967; Raymond 1969; Van Wijk 1993), and variation may turn out to be negligible when truncated at levels of maturity or heading date (Raymond 1969). Variation in digestibility between cultivars still remaining after reproductive growth may be a result of inherent differences between plants (Walters et al. 1967).

Wrightson Seeds have demonstrated an average organic matter digestibility advantage of 2.6% over Yatsyn 1 (Appendix 1.1). A pooled analysis of six cutting trials (Wrightson Seeds unpublished data), over four consecutive years and at two different trial sites also showed a significant advantage of 82.6 vs $80.5 \pm 0.30\%$ ($P < 0.005$) for Aries HD and Yatsyn 1 pastures. Each cutting trial was designed as a randomised block design with four replicate plots of 3.0 m x 0.75 m in size. At each monthly sampling date (November to February), a strip of herbage 3.0 m by 0.45 m was removed from the centre of each plot and analysed for *in vitro* organic matter digestibility. Sheep then grazed plots to a height just below the mown strip. Management of plots also included regular application of fertiliser and watering to promote vegetative and leafy growth.

In the first continuously stocked experiment the *in vitro* organic matter digestibility from herbage cut to ground level (Plate 8.1) and from pluck samples to simulate herbage selected did not differ between cultivars. Estimates of nitrogen and neutral detergent fibre were also similar. The nutritive value of pasture depends upon the management system adopted (Dent et al. 1967) and changes in management can produce changes in morphology and tiller development (Hodgson 1981; Wilson 1981; Hutchinson and Clements 1987). Grazing leaves swards more variable than those which have been defoliated by mowing machines (Morley 1978). Under continuous stocking management the herbage is continually available to the animal and there will be a continuous turnover of leaves, stems and dead material. In contrast, under intermittent grazing or cutting there is greater synchronisation of herbage accumulation and removal. Therefore it was possible that the lack of any significant contrast in organic matter digestibility in Experiments 1 and 2 was due to contrasts in pasture management with the original Wrightson Seeds work.

It was decided to use cages in Experiment 3 (Chapter 5) to better simulate the intermittent cutting management used by Wrightson Seeds (Plate 8.2). A similar contrast in organic matter digestibility resulted, although this difference was not statistically significant (79.4 vs $77.6 \pm 2.37\%$, $P = 0.6346$). To further evaluate the digestibility of regrowth, rotational grazing was used in the final grazing experiment (Chapter 7). It was anticipated that leader lambs would test the nutritional value of the pasture after a four-week regrowth period. However, no contrast in organic matter digestibility was detected from herbage cut to ground level, or to grazing height, or from oesophageal fistulate samples using rotational grazing management.

Plate 8.1 Herbage cut sample to ground level.



Plate 8.2 Herbage regrowth under cages before cutting to grazing height.



Estimates of mean organic matter digestibility pooled over the four grazing experiments are shown in Table 8.3. The pooled analysis of variance used experiments as replicates. The *in vitro* organic matter digestibility of herbage sampled by oesophageal fistulated sheep was higher in Aries HD than in Yatsyn 1 (77.3 vs $75.2 \pm 0.52\%$), approaching significance at the 5% level of probability. Because the oesophageal fistulated sheep grazed every plot the variation between animals was accounted for in the analysis of variance, reducing the coefficient of variation (between 3 - 8%) and providing a robust experimental design in which to compare organic matter digestibility. No other pooled comparison of organic matter digestibility over the four experiments approached significance.

Leaf and stem proportions of the grass plant can vary considerably in digestibility. Grazing animals will select certain parts in preference to other parts (Clements 1970; Snaydon 1979; Clark 1993), and the diet may differ greatly from that of the herbage on offer (Snaydon 1979). Therefore, the degree of selective grazing can modify the effects of differences between cultivars (Minson et al. 1960; Clements 1970). The overall diet of the animal will be improved to a degree dependent on the plant improvement and its proportion and accessibility in the sward (Hutchinson and Clements 1987; Clark and Wilson 1993). Ewes have been found to discriminate between vegetative and reproductive tillers of cocksfoot, with a trade off between quality (vegetative) and quantity (reproductive) (Prache et al. 1997). Clark (1993) reported smaller differences in digestibility between cultivars in the diet selected by sheep as opposed to whole plant digestibility.

Table 8.3 Mean *in vitro* organic matter digestibility (%) over four grazing experiments 1995 to 1998.

Measurement technique	n/trt mean	Aries HD	Yatsyn 1	SEM	P
Cut sample	4	62.3	62.2	0.24	0.7873
Plucked sample	3	69.6	69.9	0.15	0.2354
Cage sample	2	72.2	70.5	0.71	0.3385
Oesophageal fistulate sample	4	77.3	75.2	0.52	0.0637

Chapter 4 reported an evaluation of the nutritive value of the cultivars when endophyte alkaloids were absent. It was found that the proportion of leaf was significantly greater in Aries HD than in Yatsyn 1 pasture, perhaps reflecting a different rate of tiller maturity. Ewes grazing Aries HD had higher liveweight gains in September ($94 \text{ vs } 56 \pm 14.5 \text{ g/day}$) than ewes grazing Yatsyn 1 pastures, resulting from a greater herbage intake ($1.72 \text{ vs } 1.37 \pm 0.038 \text{ kg DM/day}$). It has been shown that the digestibility of stem declines at a much faster rate than that of the leaf (Tilley and Terry 1964). The higher leaf content of Aries HD ryegrass in this experiment was not reflected in higher organic matter digestibility of either whole sward samples, plucked samples or oesophageal fistulate samples. However, leaf is eaten in greater quantities than stem, even when there is no difference in digestibility (Minson 1990). Laredo and Minson (1973) reported that the intake of leaf was 46% higher than that of stem in tropical grasses, despite similar digestibility. The proportion of leaf in Aries HD and Yatsyn 1 pastures was the same in the three summer weaned lamb experiments.

The objective of the tiller demography observations (Chapter 6) was to determine if there were any differences in the onset, duration and intensity of reproductive development and the relative patterns of survival of tillers between Aries HD and Yatsyn 1. Cultivar differences in reproductive growth can result from differences in the proportions of vernalised tillers, the timing of stem elongation, or in the growth rates of reproductive tillers (Hunt and Mortimer 1982). Wilkins (1995) reported that differences

among perennial ryegrass varieties in leaf content were primarily because of differences in the proportion of flowering tillers. Therefore breeding for improved vegetative growth may indirectly lead to reduction in the proportion of reproductive tillers and a consequent increase in the leaf content of herbage at some harvests (Wilkins 1995). Seedheads first appeared in mid November for both cultivars. Aries HD appeared to have a more intense period of inflorescence initially and then a lower proportion of reproductive tillers after January than Yatsyn 1. Aries HD has been observed to have less aftermath heading than Yatsyn 1 (M. N. Norriss, personal communication). The proportion of elongating and reproductive tillers appeared to be lower on average in Aries HD than Yatsyn 1, but these differences were small and non-significant.

Under rotational grazing management (Chapter 7) Aries HD also appeared to have a greater proportion of vegetative tillers during November and December, although again the difference was not statistically significant (65.9 vs $55.54 \pm 4.70\%$, $P = 0.1539$). Small apparent differences in tiller reproductive behaviour were not translated into any significant difference in nutritive value between the two cultivars. Aries HD began flowering at the same time as Yatsyn 1 (Figure 6.4) suggesting that the difference in leaf content was not due to Aries HD having a slower rate of development. There were no obvious differences in tiller death rate between the two cultivars. Aries HD and Yatsyn 1 cultivars had similar tiller survival patterns to Grassland Nui (Korte 1986). Few tillers died until mid December, after which time survival approximated an exponential decay curve.

8.5 EFFECTS DUE TO RYEGRASS/ENDOPHYTE ASSOCIATION

Both Aries HD and Yatsyn 1 cultivars were infected with the same wild-type endophyte, and had a high level of infection (> 90% infected tillers). The percentage of endophyte infected tillers was assessed in every summer experiment (Latch and Christensen 1985). and was always the same between cultivars.

8.5.1 Digestibility

It is unclear whether the dominant effect of endophyte on liveweight change is a direct toxic effect on the animal or a secondary effect of reduced herbage intake through lower palatability or reduced appetite (Fletcher 1993a). Eerens et al. (1998) has reported that there was no difference in the chemical composition and *in vitro* organic matter digestibility between endophyte-infected and endophyte-free pastures in Gore, South Island over five years. Conroy et al. (1992) reported that high-endophyte Ellett was significantly more digestible than low-endophyte Ellett during September to late November. Cultivar integrity requires verification that characteristics such as date of maturity and seasonal growth pattern are not changed by the presence of endophyte (Fletcher and Easton 1997). Selecting a cultivar for higher digestibility will not lead to an improvement in animal performance if the plant is seriously deficient in an essential element, or is susceptible to a toxin producing organism (Hacker 1982).

8.5.2 Ryegrass staggers

Ideal conditions for the development of staggers follow extended warm dry periods where there has been a build-up of dead material in the base of the sward, and animals are forced to graze into the base (Fletcher and Easton 1997). In New Zealand these conditions are likely to occur in late January to March, but vary from year to year and may not occur at all in some years (Fletcher and Easton 1997). In Experiment 1 (Chapter 3) lambs were observed with clinical staggers from late January until early March. Peak lolitrem B concentrations were detected on 21/2/96 at 1.63 vs 1.56 ppm for Aries HD and Yatsyn 1 samples respectively. Symptoms of ryegrass staggers are

unlikely to be evident unless lolitrem B levels are greater than 2 ppm in pastures (di Menna et al. 1992; Blythe 1993). In this study herbage cut samples were analysed at monthly intervals for alkaloid concentration so it was probable that the peak of lolitrem B was missed. The mean proportion of lambs with severe staggers (% of lambs carried to weighing) was 15% in Aries HD treatments compared to 29% in Yatsyn 1 treatments. Ryegrass staggers may impair performance by reducing intake due to impaired grazing ability or due to a direct toxic effect (Fletcher 1993a).

Lolitrem B has been regarded as the major alkaloid responsible for staggers (Gallagher et al. 1984), but levels detected in the two cultivars were similar. Ergovaline concentration in Aries HD samples were consistently half that of Yatsyn 1 samples. Strain/host combinations of *Neotyphodium lolii* endophytes in perennial ryegrass have been shown to vary in their production of ergovaline (Easton et al. 1993). Piper and Fletcher (1990) concluded that ergovaline was not involved with the staggers syndrome. However, it is possible that tremorgenic alkaloids may act synergistically with other endophyte/ryegrass alkaloids in the incidence of staggers (Gallagher et al. 1977; Fletcher et al. 1993a; Clarke et al. 1996). Ergovaline has been associated with reduced liveweight gain in animals grazing perennial ryegrass (Piper and Fletcher 1990; Fletcher et al. 1991; Fletcher and Sutherland 1993). There was no difference in organic matter digestibility or herbage intake between the two cultivars, but a significant difference in liveweight gain. It was concluded from this experiment that the higher performance achieved on Aries HD pasture than on Yatsyn 1 pasture reflected the interrelated effects of alkaloid concentration and ryegrass staggers.

In Experiment 3 (Chapter 5) there was a low incidence of ryegrass staggers. Pastures were sprinkler-irrigated throughout the experiment to promote green vegetative growth and weather conditions were mild with consistent rainfall. Only 9% of Yatsyn 1 lambs and 0% of the Aries HD lambs were carried to weighing from late February to early March. Lolitrem B levels did not exceed 1.4 ppm and ergovaline concentrations were undetectable. In addition *Poa annua* contributed 43% of live herbage during this experiment and may have diluted the intake of alkaloid toxins. It was concluded that lambs had a lower alkaloid challenge than in the previous summer, and this was

reflected in the low incidence of staggers. There was again no difference in organic matter digestibility between the two cultivars. This lack of contrast in either endophyte alkaloid concentration or nutritive value was reflected in no difference in lamb performance.

The final grazing experiment (Chapter 7) was designed to assess both the nutritive value of the pasture and the alkaloid challenge imposed by the respective endophyte/cultivar associations. Lambs were rotationally grazed in a leader-follower sequence. The leader lambs grazed fresh regrowth while the followers grazed the residue left behind by the leaders. It was anticipated that the follower groups would be forced to graze into the base of the sward containing the highest concentration of endophyte alkaloids, while the leader groups would graze fresh regrowth of low alkaloid challenge. As expected, follower lambs showed clinical signs of staggering over January. In mid-February there was a sharp rise in the incidence and severity of staggers, coinciding with increasing levels of lolitrem B. All lambs irrespective of treatment developed severe staggers. Lolitrem B levels were three times higher than in the previous two summers.

On March 9 lambs were removed from toxic pasture and fed lucerne hay until they were reintroduced on 23 March. Lambs 'recovered' in less than one week once removed, demonstrating the effectiveness of feeding an alternative forage, but were again removed on 7 April due to recurrence of severe staggers (mean score 4.7). The surrounding research farm experienced high lamb losses through misadventure and disorders relating to lambs being cast for extended periods of time. Follower lambs were more severely affected by staggers, but this observation was not mirrored in significantly higher lolitrem B concentrations. Lolitrem B is concentrated towards the base of the plant, particularly in the senescent leaf sheath (Davies et al. 1993b). Pownall et al. (1993b) reported that ryegrass staggers severity increased as pasture horizons were progressively removed using a leader-follower grazing system. Herbage samples for alkaloid analyses were cut to ground level and did not reflect the diet selected by the lambs. Samples cut to grazing height may have better reflected the concentration of alkaloids ingested by lambs. Cosgrove (1996) reported that leader and follower pasture grazed by cattle did not differ significantly in lolitrem B concentration.

Due to the cost of alkaloid analysis it was not feasible to analyse samples cut to grazing height in the current study programme.

Yatsyn 1 lambs had significantly higher staggers severity scores than Aries HD lambs. Lolitrem B concentration did not differ significantly between cultivars but, as in experiment 1 (Chapter 3), ergovaline concentration in Aries HD samples was consistently half those of Yatsyn 1 samples. Higher ergovaline concentrations may have been acting synergistically with lolitrem B concentrations to increase the severity of staggers observed in lambs grazing Yatsyn 1 pastures. The difference in staggers severity and ergovaline concentration between cultivars was not translated into any significant difference in lamb performance, and it is possible that differences could have been masked by the severe and debilitating effects of staggers in this study. There were no contrasts in herbage nutritive value between Aries HD and Yatsyn 1 pastures in this study.

8.5.3 Heat stress

Ergopeptine alkaloids and in particular ergovaline can cause hyperthermia and depressed serum prolactin levels in sheep during summer (Fletcher and Barrell 1984; Fletcher 1993b). Results from Experiment 1 (Chapter 3) indicated that the Aries HD/endophyte association may produce less ergovaline than the Yatsyn 1/endophyte association, so in Experiment 3 rectal temperature and serum prolactin concentration were measured. However, ergovaline concentrations were barely detectable in herbage samples and ambient temperatures were not high enough to evoke either heat stress symptoms or differences between cultivars. The final experiment (Experiment 4) was designed to specifically measure rectal temperature, respiration rate and serum prolactin concentration. Lambs were housed in a shed during measurement to ensure stable environmental sampling conditions and high air temperatures.

Respiration rate, which is a sensitive measure of heat stress, was higher in Yatsyn 1 lambs, possibly reflecting the significantly greater ergovaline concentration in Yatsyn 1 pasture. Respiration rate showed a curvilinear response with increasing ambient

temperature, reaching a maximum of about 80 respirations/minute at 31 °C in this study (Figure 7.6). Rectal temperature did not differ between treatments in the range of ambient temperatures experienced, and would perhaps be a more sensitive measure of heat stress in ambient temperatures over 31 °C where respiration rate is already at a maximum level. Serum prolactin concentration can be a good indicator of intoxication with ergopeptine alkaloids such as ergovaline (Fletcher and Easton 1997). Ergovaline concentration was 28% higher in follower herbage samples, possibly explaining the significantly reduced serum prolactin concentration and respiration rates. The higher ergovaline levels may also have acted synergistically with lolitrem B to increase its toxicity and the severity of staggers observed in follower lambs. Results suggest that symptoms of heat stress in lambs are more severe in lambs forced to graze lower into the sward.

8.6 IMPLICATIONS

8.6.1 Potential in breeding for improved nutritive value and digestibility

There is wide agreement in the literature that selection for high organic matter digestibility is a valid breeding objective in perennial ryegrass, and a small improvement could have a significant impact on animal production systems (Section 2.2.8). However, how successful past attempts have been in increasing the feeding value of perennial ryegrass is widely argued. Selections of other forage species including bermudagrass, switchgrass and wheat grass have demonstrated improved digestibility and increased animal production (Vogel and Sleper), though the margin for improvement was much greater in these species than in perennial ryegrass because of their inherently lower digestibility. Estimates of heritability for organic matter digestibility suggest that some advance under selection can be expected in species already of high digestibility such as perennial ryegrass, particularly when at a fairly mature stage at or after flowering. Differences of about 1.25% in organic matter digestibility can be reliably detected by the *in vitro* technique (Dent et al. 1967). Any selection index for improving nutritive value must include digestibility because it integrates nutritive value components increasing the selection efficiency (Hutchinson and Clements 1987). However, a single determination of the apparent digestibility of a cultivar may not be enough to provide a basis for the exact prediction of animal performance in the complex grazing situation (Blaxter 1960).

Problems of breeding pasture plants for improved nutritive value include not taking into account cultivar x maturity interactions, selective grazing by animals, and cultivar x environment interactions (Clements 1970). Variation in digestibility achieved during the breeding process can be diluted down the line. For example, if a breeder selects for digestibility at the heading stage and with spaced plants, then the effect may be reduced for other growth stages, for swards versus spaced plants, for mixtures versus monocultures and for other environments in other years (Hutchinson and Clements 1987). Wrightson Seeds comparisons of organic matter digestibility were made from pastures that were well watered and fertilised. Samples were cut using a lawn mower

and then grazed before being allowed to regrow for one month. These evaluations have shown that it can be dangerous to extrapolate results to other situations such as continuously stocked management systems.

The factors that are most likely to affect relative performance are the age of the sward, cutting/grazing management, fertiliser use and uncontrolled variation in soil and climate (Snaydon 1979). It is unwise to assume that genotypes selected in one environment will perform well in another (Clements 1970). Aries HD has demonstrated higher organic matter digestibility than Yatsyn 1 in five trials in Christchurch and one in the Waikato (Wrightson Seeds unpublished data). Environmental factors such as drought can affect the digestibility of a forage (Hacker 1982; Vogel and Sleper 1994). However, because the experiments in this study ran over three consecutive years and at two trial sites there was a good opportunity to cover a range of environmental conditions. In Experiment 3 pastures were irrigated to maintain green leafy growth similar to conditions maintained by Wrightson Seeds in their evaluations of digestibility. Therefore it is unlikely that the environmental conditions in Palmerston North were sufficiently different to those in Christchurch or the Waikato to cause the lack of contrast in digestibility between the two cultivars. A cultivar specifically selected for higher digestibility should ideally show an improvement over a wide range of environments or regions.

Differences between cultivars in animal production are unpredictable, and are very small compared with the effects of environment and management that account for over 95% of the variation (Snaydon 1979). The variation achieved by the management imposed on perennial ryegrass will have much more influence on the quality of that grass than the advances likely by breeding (Rogers and Thomson 1970). However, an increase in the nutritive value of a pasture could increase the level of production achieved at a given level of management input.

Differences in animal performance obtained under experimental conditions may be only partially retrieved under farm conditions. Cultivar comparisons are usually sown as pure swards, with fertiliser use and under near optimal conditions. Trials may mask inherent weaknesses in cultivars which may later show up under farming management

(Reed 1994). Plants selected for improved nutritive value have to perform in complex pastures, often with nutrient limitations, and under a range of defoliation pressures (Snaydon 1979; Clark and Wilson 1993). The range in variation of components in nutritive value may not appear to be of real agronomic importance, being too small to demonstrate on a farm scale (Rogers and Thomson 1970). Therefore a difference of 2.6% in organic matter digestibility may be too small to result in any significant difference in animal performance.

Pooled estimates of mean digestibility over the four grazing experiments showed that *in vitro* organic matter digestibility did not differ between Aries HD and Yatsyn 1 from samples cut to ground level or from hand plucked samples. There was a significant contrast in digestibility of 2% from herbage sampled by oesophageal fistulate sheep and an apparent difference in samples cut to grazing height, indicating that management technique affects digestibility. In addition, herbage intake was on average significantly higher on Aries HD pasture than on Yatsyn 1 pasture. Contrasts between the cultivars were not consistent or sufficiently large to be translated into significant differences in sheep performance. However, a cumulative improvement over time in digestibility by plant breeders could result in an exploitable increase in nutritive value (Rogers and Thomson 1970).

Benefits from plants having improved nutritive value cannot be realised unless they can be successfully established and maintained through fertiliser policies and grazing management (Clark and Wilson 1993). There may be no point in improving the digestibility of a given species or even a part of it, if the environment and stocking rate do not ensure that the animal eats that species or part (Clements 1970). Pasture utilisation should remain at levels that give stable long-term yield and botanical composition (Clark and Wilson 1993). The ingestion of any one species, bred with improved nutritive value, can not be assumed (Hutchinson and Clements 1987).

An improvement in nutritive value may reduce the number of animals supported per unit area if it results in an increase in intake per animal or an associated reduction in herbage production (Hodgson 1981). There is no evidence to suggest that herbage

production was less on Aries HD pasture than on Yatsyn 1 pasture. Comparative herbage yield data was not collected in these experiments, but the carrying capacity of plots (mean animals/plot) did not differ between cultivars at any stage. Drymatter production from Aries HD collected by Wrightson Seeds shows good summer and autumn growth comparable to Yatsyn 1 (Appendix 7.1).

8.6.2 Potential value of Aries HD perennial ryegrass

No single species or cultivar is likely to be superior under all conditions or in every season. Aries HD has been promoted to be significantly higher in organic matter digestibility than other commercial varieties (Figure 8.1). This series of evaluations has not shown a consistent improvement in digestibility. There is evidence which suggests that Aries HD plants have finer tillers in a higher population density than Yatsyn 1, which could aid in persistence under hard grazing. The tiller demography study also indicated that Aries HD has a lower proportion of secondary reproductive tillers (lower aftermath heading). However, small differences in reproductive behaviour were not translated into any significant difference in organic matter digestibility. Aries HD has demonstrated animal performance or feeding value similar to, or better than Yatsyn 1, which is a proven industry standard among cultivars of perennial ryegrass. In addition, Aries HD shows lower potential risk from endophyte alkaloids, which can adversely affect the health and production of grazing sheep. It can therefore be concluded that the feeding value of Aries HD is equal to, or better than Yatsyn 1 perennial ryegrass.

Figure 8.1 Aries HD perennial ryegrass promotional characteristics (Wrightson Seeds 1995: Appendix 7.1).

-
- ♣ Be the first perennial ryegrass to be bred specifically for high digestibility.
 - ♣ Have dense fine leaves to aid in persistence under hard grazing giving the farmer flexible grazing options.
 - ♣ Have low aftermath heading (less seedheads) to keep the pasture leafy and extend the quality growing period of pasture for stock.
 - ♣ Have good year round growth with emphasis on summer quality providing feed for a range of stock types.
-

8.6.3 Potential value of novel endophytes

Synthetic ryegrass/endophyte associations have been developed which do not produce lolitrem B or ergovaline (Fletcher and Easton 1997). Of concern with the new associations will be the risk of reduced spectra of pesticidal activity resulting in less plant protection (Siegal 1993). Ball and Prestidge (1993) warn that developing an endophyte strain that retains the beneficial aspects (insect resistance) and eliminates the adverse effects (animal toxicity) may not eliminate all problems as these effects may be interrelated. For example, an ergot alkaloid (ergotamine) deterred adult black beetle from feeding on ryegrass at 1 ppm.

Novel endophytes may have limited impact on New Zealand farms. The use of the new ryegrasses/endophyte selections will be restricted to relatively small areas of New Zealand where it is physically and economically feasible to renew pastures. Some of these pastures will always be faced with some degree of stock health problems, until suitable and cost-effective animal remedies can be developed (Garthwaite 1995; Fletcher et al. 1996). Existing ryegrass and buried seed of natural endophyte associations must be eliminated before resowing with the new ryegrass (Garthwaite 1995; Fletcher and Easton 1997). New swards must also be maintained free of

contamination by toxic natural associations. Even 10-20% contamination by natural associations under adverse conditions could negate the benefits of a new toxin-free association (Fletcher and Easton 1997). Extension officers and farmers require education on how to ensure maximum benefits from new synthetic grass/endophyte associations (Fletcher and Easton 1997). New grass/endophyte associations could be utilised during critical periods and by more sensitive classes of stock, with toxic pasture being grazed in cooler weather to avoid accumulation of herbage (Fletcher and Easton 1997).

8.8.4 Importance of cultivar evaluations

Attempts to breed cultivars for increased nutritive value or digestibility must be validated in animal grazing evaluations. This animal evaluation should ideally occur before commercial release. There have been very few long term comparisons of animal production from contrasting cultivars in New Zealand. Issues such as endophyte alkaloid production with associated health problems and compatibility with companion clover species should be examined over a number of seasons. Farmers require sound animal performance data to enable them to decide not only which cultivar of perennial ryegrass to use, but the choice of endophyte strain and infection level. Information on liveweight gain, ryegrass staggers incidence and alkaloid concentrations from Chapter 3 have been included in a booklet for farmers (Appendix 1.1). The success of novel ryegrass/endophyte associations relies on research to establish guidelines for successful establishment and management to maintain pastures by farmers.

8.7 CONCLUSIONS

The following general conclusions can be drawn from this series of experiments:

- 1) The nutritive value (*in vitro* organic matter digestibility, neutral detergent fibre and nitrogen content) of Aries HD was similar to that of Yatsyn 1 perennial ryegrass, from herbage samples cut to ground level, from plucked samples and from cage cut samples under both continuous grazing and rotational grazing management. Evidence by Wrightson Seeds of a higher level of organic matter digestibility in Aries HD than in Yatsyn 1 swards under comparable management were not substantiated, though *in vitro* organic matter digestibility pooled across experiments was higher in Aries HD than in Yatsyn 1 pasture from samples selected by oesophageal fistulated sheep.
- 2) Aries HD swards have a higher density of finer tillers than Yatsyn 1. The two cultivars had similar tiller survival patterns. Aries HD appeared to have a more rapid onset of initial flowering but then a lower proportion of secondary reproductive tillers. There is some evidence that the proportion of vegetative tillers on average was greater in Aries HD than in Yatsyn 1 swards. Leaf percentage was significantly higher in Aries HD swards over spring, possibly contributing to the higher herbage intake and liveweight gain of ewes grazing Aries HD during a period of low alkaloid risk. Small apparent differences in the reproductive behaviour were not translated into any significant difference in nutritive value of the two cultivars.
- 3) Mean herbage intake was higher in sheep grazing Aries HD than in sheep grazing Yatsyn 1 perennial ryegrass. Grazing behaviour was similar between the two cultivars of perennial ryegrass.
- 4) The three summer experiments covered a range of environmental conditions which maximised the opportunity to investigate the effect of endophyte. Alkaloid challenge ranged from minimal (Experiment 3) to moderate

(Experiment 1) to extreme (Experiment 4) and was the major determinant of the animal responses.

- 5) Aries HD in association with endophyte consistently produced half the concentration of the alkaloid ergovaline as did Yatsyn 1 in association with endophyte. Respiration rate, as an indicator of heat stress, was higher in lambs grazing Yatsyn 1 pasture (Experiment 4).
- 6) Sheep grazing Yatsyn 1 were more severely affected by ryegrass staggers than sheep grazing Aries HD perennial ryegrass, although levels of lolitrem B were the same. Higher ergovaline concentrations in Yatsyn 1 pasture may have acted synergistically with lolitrem B to increase its toxicity and the severity of staggers observed in lambs grazing Yatsyn 1 swards.
- 7) The leader/follower grazing regime (Experiment 4) created contrasts in sward conditions resulting in substantial reductions in *in vitro* organic matter digestibility and liveweight gain of lambs grazing follower swards. Heat stress symptoms were more severe in lambs forced to graze lower into the base of the sward as measured by significantly reduced serum prolactin levels and respiration rates in follower lambs, possibly reflecting greater ergovaline intoxication.
- 8) The feeding value of Aries HD perennial ryegrass as determined by the liveweight gain of grazing sheep was superior to that of Yatsyn 1 in two evaluations and the same in two evaluations. Differences in animal performance could not be attributed to differences in herbage nutritive value but reflected the effects of endophyte.
- 9) Grazing management and alkaloid challenge can determine the performance of weaned lambs grazing high-endophyte ryegrass over summer and autumn. Although both Aries HD and Yatsyn 1 were infected with wild-type endophyte, they have been shown to vary in their production of ergovaline which is an

alkaloid harmful to the performance and health of grazing animals. The effect of management and alkaloid concentration is likely to have a larger impact on lamb performance than small differences in nutritive value between high-endophyte cultivars.

- 10) The outcome of these studies emphasises the need for animal evaluation experiments to assess not only nutritive value of ryegrass cultivars, but also the effects of the cultivar/endophyte association on the production and balance of alkaloids.

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APPENDICES

Appendix 1.1 Information on Aries HD, including results from Chapter 3 (Wrightson Seeds 1995).

GRASS OPTIONS

5

PERENNIAL RYEGRASS

ARIES HD

Aries HD is a versatile, perennial ryegrass bred for high digestibility. It adapts well to varying environmental conditions, performing just as well under sheep grazing in Canterbury as it does under dairy management in the Waikato. Trials show Aries HD to be significantly more digestible than existing varieties which can lead to improved intake and animal performance.



- High digestibility
- Versatile – performs well under sheep and cattle grazing
- Tolerates heavy stocking
- Good disease resistance
- Reduced endophyte toxicity

SOWING AND ESTABLISHMENT

Aries HD can be sown at 15-25kg/ha with a legume. It combines well with other components of a pasture mix (eg white and red clover, Italian ryegrass, cocksfoot, timothy and Grasslands Puna Chicory. Aries HD can tolerate hard sheep grazing without a reduction in the number of Aries HD plants in the sward.

GRAZING MANAGEMENT

Aries HD is a utility grass suited to a range of grazing systems. It performs well under rotational grazing by both cattle and sheep and also persists well if hard grazed or set stocking by sheep.

ARIES HD INFORMATION

Quick Reference Comparative Guide

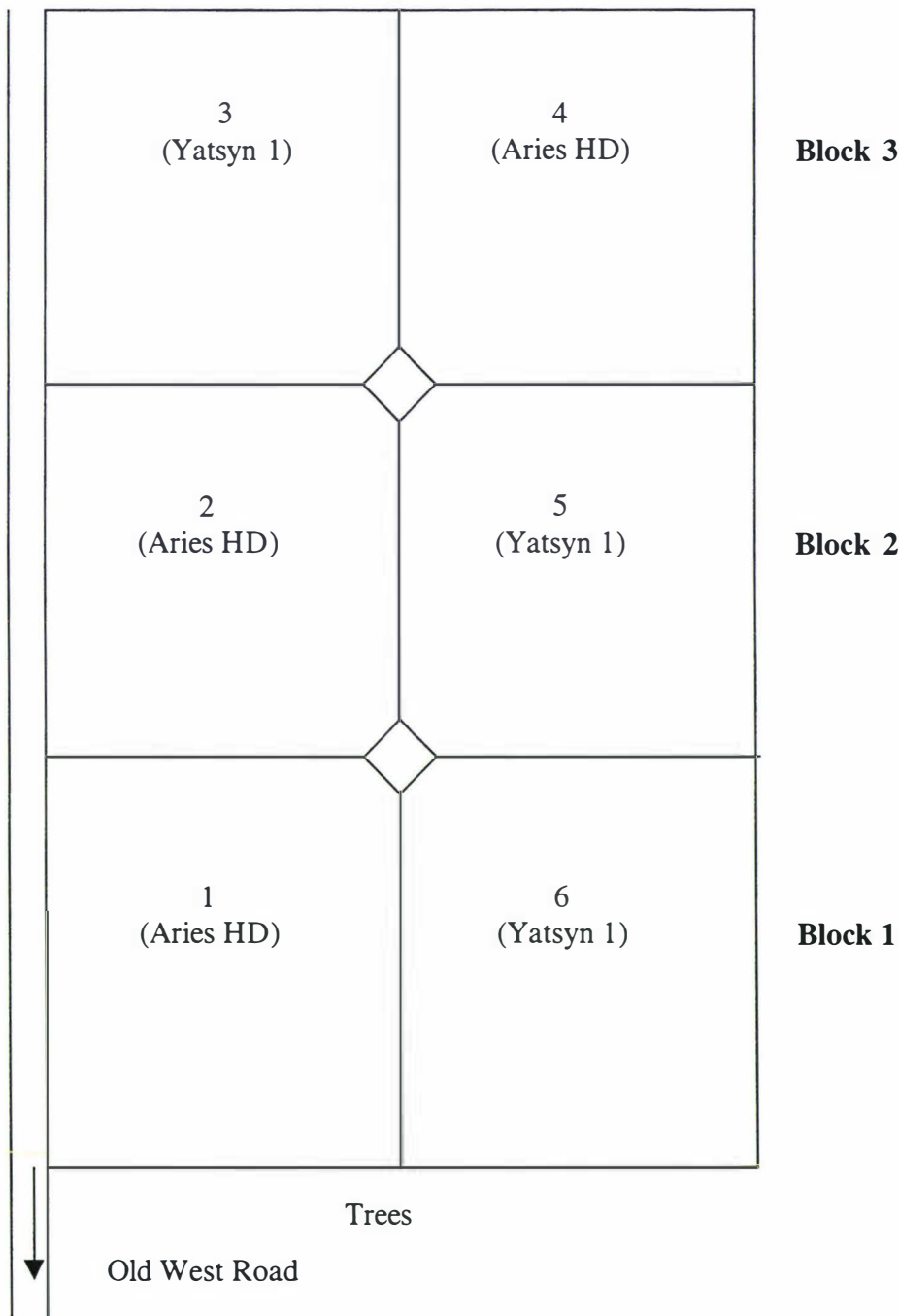
	ARIES HD	YATSYN 1
Yield	99	100
<i>Evaluations show Aries HD yields at a similar level to other leading perennial ryegrasses</i>		
Digestibility	87.2%	84.6%
<i>Aries HD increased digestibility leads to greater feed intake and more rapid growth rates</i>		
Endophyte Status	90.8	91.7
<i>Aries HD contains similar levels of perennial endophyte to other ryegrasses</i>		
Ergovaline ppm	0.23	0.42
<i>The lower the figure the better, high levels affect blood flow and lower feed intake</i>		
Ryegrass Staggers	15%	29%
<i>A Lamb grazing trial confirms reduced negative endophyte effect</i>		
Liveweight Gains	104 gms/day	84 gms/day
<i>The same trial confirms the positive growth from grazing Aries HD</i>		

Ref: 1995/96 'Summer Finishing Lamb Experiment' – Massey University and Kimihia Research Centre

Appendix 2.1 The system of scoring used to assess severity of ryegrass staggers symptoms (Keogh 1973).

Score	Description of symptoms
0	No symptoms.
1	Slight trembling of neck, shoulders, and flank muscles after hard exercise (400 m run).
2	Marked trembling of neck, shoulders and flank muscles, and shaking of head after hard exercise, but no lack of co-ordination.
3	Marked trembling of general musculature and head shaking; some lack of co-ordination of movement and impaired vision while running.
4	Muscle tremors and head shaking after a short run (< 30 m) or sudden disturbance; continued exercise elicits a marked lack of co-ordination resulting in a characteristic staggering gait which normally ends with the animal falling down; a short period of moderate to severe muscular spasms follows, after which the animal is able to regain its feed and walk off.
5	Severe muscle tremors elicited by slight disturbance or exercise (< 10 m rapid movement) which invariably result in staggering and collapse in a severe tetanic spasm which may last up to 20 minutes in very bad cases.

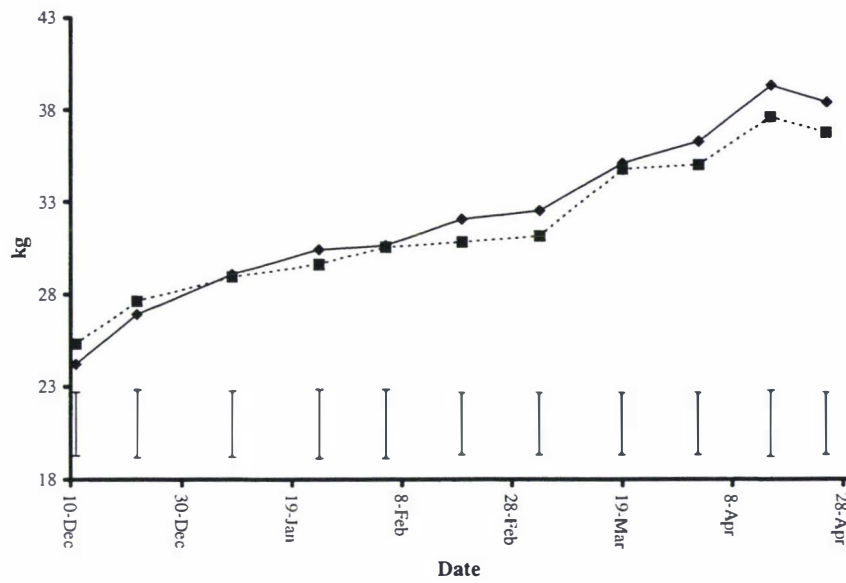
Appendix 3.2 Trial design of Experiment 1, 2 and 3 (Chapter 3, 4 and 5) at Haurongo
Sheep and Beef Research Unit, Massey University, Palmerston North.



Appendix 3.3 Monthly rainfall, soil temperature (10 cm), air temperature and maximum air temperature during Experiment 1 (Chapter 3) from 11/12/95 to 25/4/96 at AgResearch Palmerston North.

Month	Monthly mean rainfall (mm)	Monthly mean soil temperature (°C)	Monthly mean air temperature (°C)	Monthly mean maximum air temperature (°C)
December	101.0	18.0	17.6	21.9
January	50.5	19.4	18.8	23.0
February	128.7	18.5	18.3	22.8
March	79.1	15.5	15.3	20.0
April	160.4	15.0	15.3	19.0

Appendix 3.4 Liveweight (kg) over time of lambs grazing Aries HD (◆) and Yatsyn 1 (■) pasture in Experiment 1 (Chapter 3).



Appendix 3.5 Nutritive value of Aries HD and Yatsyn 1 pastures in Experiment 1 (Chapter 3) from herbage cut samples to ground level and bulked pluck samples (fortnightly) corresponding to the same period.

In vitro organic matter digestibility (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
27/12/95	65.2	67.9	December	70.5	72.0
25/1/96	56.5	57.4	January	58.2	60.7
21/2/96	51.2	49.9	February	63.2	60.9
20/3/96	59.2	59.0	March	70.4	70.0
19/4/96	61.2	60.2	April	69.3	72.0

Neutral detergent fibre content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
27/12/95	54.4	52.4	December	51.7	51.8
25/1/96	60.0	60.0	January	62.4	60.0
21/2/96	64.4	66.0	February	55.4	57.1
20/3/96	60.2	59.8	March	49.7	50.0
19/4/96	60.6	62.1	April	50.4	48.7

Nitrogen content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
27/12/95	1.48	1.71	December	2.20	2.42
25/1/96	1.42	1.34	January	1.84	2.05
21/2/96	1.78	1.59	February	2.39	2.42
20/3/96	2.25	2.19	March	3.43	3.25
19/4/96	2.16	1.90	April	3.38	3.57

Appendix 4.1 Monthly rainfall, soil temperature (10 cm), air temperature and maximum air temperature during Experiment 2 (Chapter 4) from 3/9/96 to 1/12/96 at AgResearch Palmerston North.

Month	Monthly mean rainfall (mm)	Monthly mean soil temperature (°C)	Monthly mean air temperature (°C)	Monthly mean maximum air temperature (°C)
September	102.8	11.5	12.4	16.8
October	95.6	13.7	13.1	17.2
November	100.5	14.4	13.2	17.1
December	91.1	16.7	15.9	20.2

Appendix 4.2 Nutritive value of Aries HD and Yatsyn 1 pastures in experiment 2 (Chapter 4) from herbage cut samples to ground level and bulked pluck samples (fortnightly) corresponding to the same period.

In vitro organic matter digestibility (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
6/9/96	77.1	76.8	September	76.7	77.8
4/10/96	65.1	63.1	October	72.6	72.7
1/11/96	62.1	59.6	November	72.3	71.6
28/11/96	68.9	69.2			

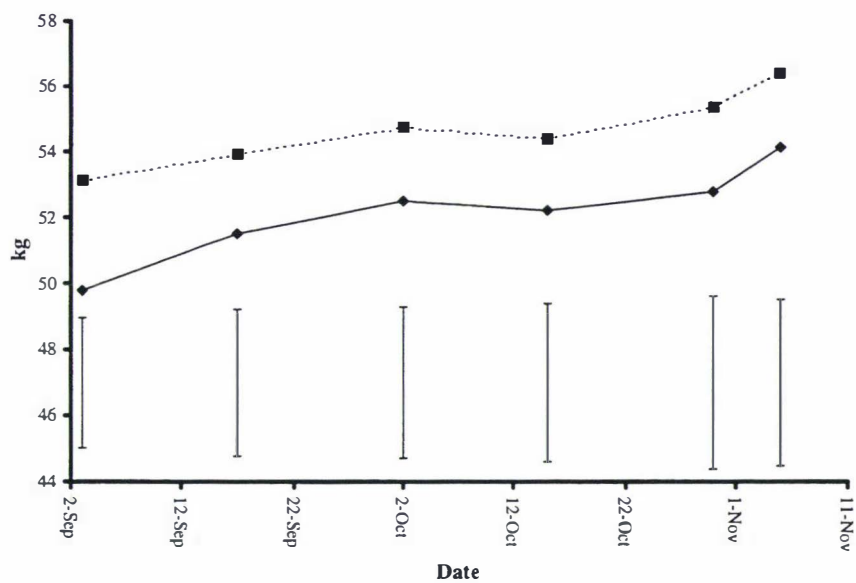
Neutral detergent fibre content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
6/9/96	50.0	50.6	September	42.6	41.8
4/10/96	55.4	59.8	October	46.9	45.9
1/11/96	58.3	58.5	November	41.1	42.0
28/11/96	50.7	53.0			

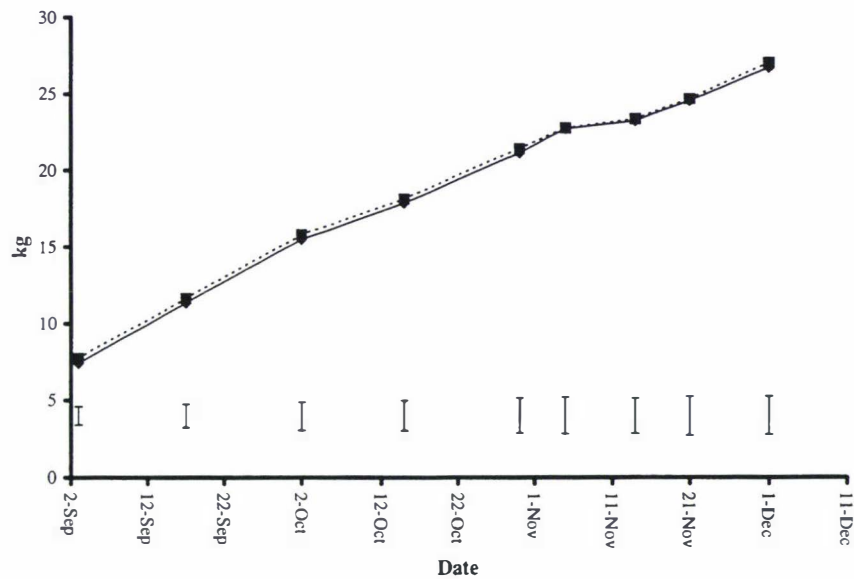
Nitrogen content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
6/9/96	2.51	2.44	September	3.46	3.74
4/10/96	2.32	1.87	October	3.37	3.27
1/11/96	2.04	2.12	November	4.30	4.00
28/11/96	2.27	1.95			

Appendix 4.3 Liveweight (kg) over time of ewes grazing Aries HD (◆) and Yatsyn 1 (■) pasture in Experiment 2 (Chapter 4).



Appendix 4.4 Liveweight (kg) over time of lambs grazing Aries HD (◆) and Yatsyn 1 (■) pasture in Experiment 2 (Chapter 4).



Appendix 5.1 Monthly rainfall, soil temperature (10 cm), air temperature and maximum air temperature during Experiment 3 from 2/12/96 to 12/3/97 at AgResearch Palmerston North.

Month	Monthly mean rainfall (mm)	Monthly mean soil temperature (°C)	Monthly mean air temperature (°C)	Monthly mean maximum air temperature (°C)
December	91.1	16.7	15.9	20.2
January	68.0	17.6	15.9	21.0
February	58.0	17.7	16.4	22.0
March	68.1	15.8	18.2	19.9

Appendix 5.2 Nutritive value of Aries HD and Yatsyn 1 pastures in Experiment 3 (Chapter 5) from herbage cut samples to ground level and bulked pluck samples (fortnightly) corresponding to the same period.

In vitro organic matter digestibility (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
28/11/96	68.9	69.2	December	75.0	75.5
30/12/96	61.5	61.5	January	66.7	71.0
24/1/97	61.2	61.2	February	68.1	65.0
24/2/97	58.4	59.3	March	64.1	63.3
27/3/97	56.9	56.7			

Neutral detergent fibre content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
28/11/96	50.7	53.0	December	44.1	43.5
30/12/96	54.8	55.9	January	48.6	46.3
24/1/97	54.5	54.3	February	48.9	49.5
24/2/97	57.6	56.9	March	53.5	54.1
27/3/97	57.5	56.7			

Nitrogen content (%)

Herbage cut samples			Pluck samples		
Date	Aries HD	Yatsyn 1	Period	Aries HD	Yatsyn 1
28/11/96	2.27	1.95	December	3.45	3.45
30/12/96	1.91	1.83	January	2.83	2.90
24/1/97	1.94	1.87	February	2.69	2.66
24/2/97	2.25	2.33	March	3.04	2.92
27/3/97	2.34	2.42			

Appendix 5.3 Nutritive value of Aries HD and Yatsyn 1 pasture in Experiment 3 (Chapter 5) under cages cut to grazing height (4 cm) and allowed to regrow for 4 weeks.

In vitro organic matter digestibility (%)

Cut	Date	Aries HD	Yatsyn 1
1	14/11/96	82.3	80.5
2	11/12/96	76.3	72.7
3	8/1/97	78.7	78.1
4	5/2/97	80.4	79.3
5	4/3/97	73.3	74.3
6	2/4/97	74.6	76.5

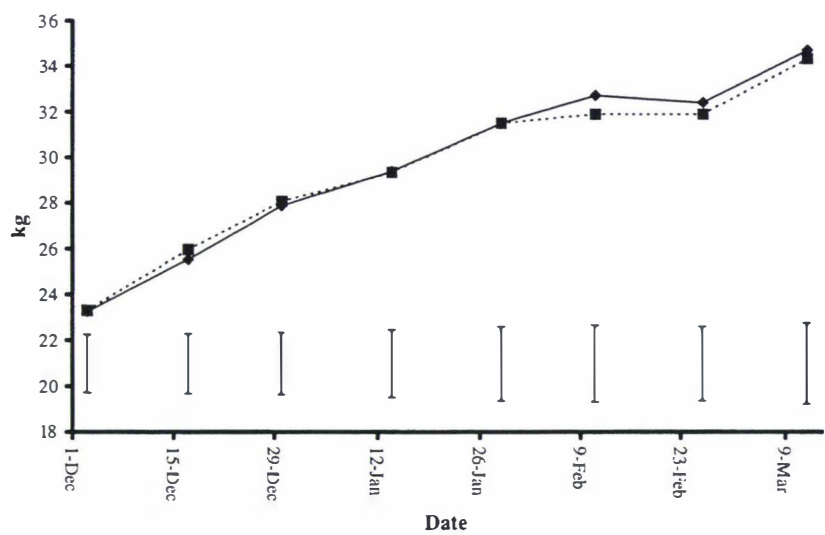
Neutral detergent fibre content (%)

Cut	Date	Aries HD	Yatsyn 1
1	14/11/96	40.5	40.6
2	11/12/96	46.9	49.4
3	8/1/97	45.0	46.9
4	5/2/97	43.1	44.9
5	4/3/97	45.1	46.1
6	2/4/97	45.1	45.9

Nitrogen content (%)

Cut	Date	Aries HD	Yatsyn 1
1	14/11/96	3.70	2.79
2	11/12/96	2.68	2.14
3	8/1/97	2.46	2.41
4	5/2/97	2.75	2.64
5	4/3/97	3.38	3.11
6	2/4/97	3.41	3.26

Appendix 5.4 Liveweight (kg) over time of lambs grazing Aries HD (◆) and Yatsyn 1 (■) pasture in Experiment 3 (Chapter 5).



Appendix 7.1 Wrightson Seeds promotional information for Aries HD (1995).

Aries HD #1a
To be read in conjunction
with Aries HD #1b

A G R I D A T A

ARIES

PERENNIAL RYEGRASS




The first perennial ryegrass specifically
bred for high digestibility

Aries HD Perennial Ryegrass is believed to be the first perennial ryegrass to be specifically bred for high digestibility. The aim of the breeding programme was to create a perennial ryegrass with high digestibility, and be consistent with the Animal Friendly* philosophy of pasture selection.

The breeding process involved hundreds of individual plants of **Aries** Perennial Ryegrass and adjacent plants of a standard ryegrass. These were harvested and analysed for digestibility using Near-Infrared Spectro Photometry (NIR). The results showed that there was useful variation in the digestibility of perennial ryegrass. The data were used to select elite plants that exhibited greater digestibility than the standard. The elite plants were crossed and the resultant seed line was subjected to more intensive digestibility testing.

Aftermath heading was also observed in the selection process. **Aries** Perennial Ryegrass exhibits low aftermath heading (less seedheads) after grazing, which produces a more leafy pasture during the summer period. This further enhanced the quality of the plants selected.

After the selection process and digestibility testing, the cultivar was named **Aries HD** Perennial Ryegrass.

- First perennial ryegrass to be bred specifically for high digestibility
- Dense fine leaves aids persistence under hard grazing giving you flexible grazing options
- Low aftermath heading (less seed heads) keeps the pasture leafy and extends the quality growing period of pasture for your stock
- Good year round growth with emphasis on summer quality provides feed for a range of stock types

USER GUIDE

SOWING

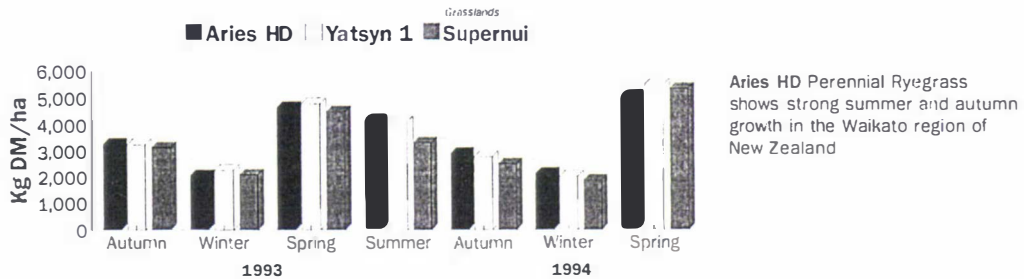
Aries HD Perennial Ryegrass can be sown as you would any other perennial ryegrass. Soil testing and careful seedbed preparation should be done to aid successful establishment. If lime is required, this should be done at the cultivation stage. **Aries HD** Perennial Ryegrass can be sown on most soil types, however, sowing rates and dates will vary from district to district. See the Sowing Guide for recommended mixtures.

ENDOPHYTE

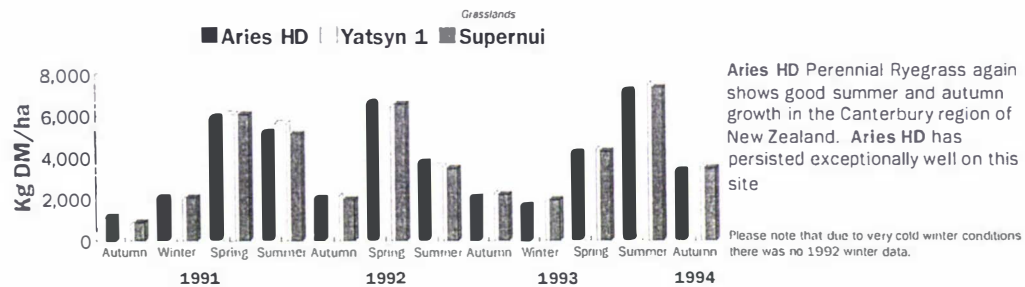
Aries HD Perennial Ryegrass is available as high endophyte only. Endophyte offers many benefits to farms prone to Argentine Stem Weevil attack and may protect pastures from other pests. However, the endophyte may affect animal health. Zero endophyte **Aries HD** Perennial Ryegrass will be available in the future.

Perennial Ryegrass Drymatter Production

1993 New Zealand, Waikato Dairy Farm Trial Drymatter Production Under Rotational Grazing



1991 New Zealand, Kimihia Research Centre Grazing Trial Drymatter Production Under Hard Rotational Sheep Grazing



DRYMATTER DEGRADABILITY

Preliminary results from drymatter degradability in the rumen of sheep shows Aries HD Perennial Ryegrass to have a rapid breakdown in the rumen. The drymatter degradability of Aries HD Perennial Ryegrass in the animal has confirmed the digestibility results obtained in the laboratory. Mr Norriss the breeder of Aries HD Perennial Ryegrass explained that when consumed feed passes out of the rumen faster it leads to increased feed intake, more rapid growth rates and considerably less drymatter required to reach animal production targets.

ENDOPHYTE

Aries HD Perennial Ryegrass contains perennial ryegrass endophyte. The endophyte produces various chemicals that can adversely affect grazing animals and protect plants from some insect attack. The most important chemicals were tested from ryegrass samples harvested from a replicated grazing trial at Kimihia Research Centre and are presented in the following table:

	Ergovaline ppm	Lolitrems ppm	Peramine ppm
Aries HD	0.23	1.8	4.0
Yatsyn 1 (control)	0.70	2.1	6.5

Average from two harvests - 20/12/94 and 18/1/95
Chemical analysis performed by AgResearch Grasslands

Ergovaline (the lower the better) causes constriction of blood vessels and reduces the ability of an animal to cool itself. This in turn can reduce the animals feed intake during warm summer conditions.

Lolitrems (the lower the better) causes perennial ryegrass staggers.

Peramine deters feeding from Argentine Stem Weevil.

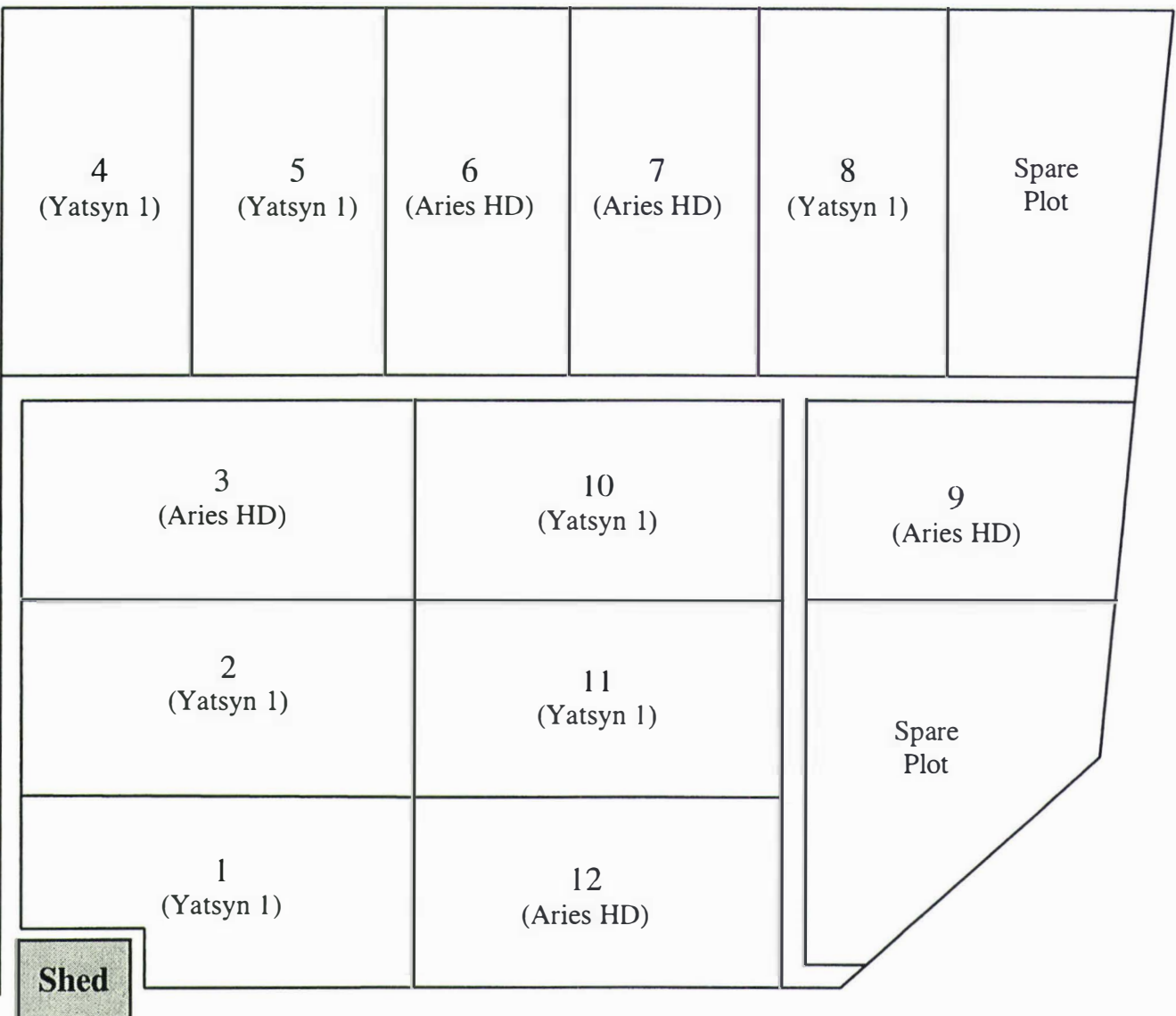
Mr Norriss suggested that Aries HD Perennial Ryegrass would produce similar levels of perennial ryegrass staggers as Yatsyn 1, but the significantly lower levels of ergovaline would lead to reduced adverse effects from this chemical.

Published June 1995



Wrightson Seeds

Appendix 7.2 Trial design of Experiment 4 at Moginie Pasture and Crop Research Unit, Massey University, Palmerston North.



Appendix 7.3 Monthly rainfall, soil temperature (10 cm), air temperature and maximum air temperature during Experiment 4 (Chapter 7) from 2/12/97 to 7/4/98 at AgResearch Palmerston North.

Month	Monthly mean rainfall (mm)	Monthly mean soil temperature (°C)	Monthly mean air temperature (°C)	Monthly mean maximum air temperature (°C)
December	103.4	17.0	15.8	20.4
January	31.7	17.9	17.6	22.5
February	61.4	20.1	20.3	25.1
March	35.6	17.6	18.3	23.1
April	72.9	14.2	15.3	19.9

Appendix 7.4 Nutritive value of Aries HD and Yatsyn 1 pastures in Experiment 4
(Chapter 7) from herbage cut samples to ground level before grazing.

In vitro organic matter digestibility (%)

Leaders			Followers		
Date	Aries HD	Yatsyn 1	Date	Aries HD	Yatsyn 1
9/12/97	64.1	65.3	16/12/97	58.2	60.9
6/1/98	69.3	66.3	13/1/98	53.3	55.2
3/2/98	59.6	62.0	10/2/98	59.8	58.7
24/2/98	55.8	55.9	3/3/98	51.4	52.8
23/3/98	54.0	53.8	31/3/98	48.8	49.5
15/4/98	64.5	60.0			
4/5/98	70.9	66.2			

Neutral detergent fibre content (%)

Leaders			Followers		
Date	Aries HD	Yatsyn 1	Date	Aries HD	Yatsyn 1
9/12/97	57.5	57.6	16/12/97	60.3	57.8
6/1/98	55.8	58.9	13/1/98	66.5	64.8
3/2/98	58.8	58.2	10/2/98	60.4	63.5
24/2/98	60.8	60.1	3/3/98	64.0	64.0
23/3/98	61.7	62.7	31/3/98	65.1	64.3
15/4/98	53.6	57.4			
4/5/98	49.9	54.1			

Nitrogen content (%)

Leaders			Followers		
Date	Aries HD	Yatsyn 1	Date	Aries HD	Yatsyn 1
9/12/97	1.55	1.88	16/12/97	1.45	1.26
6/1/98	2.62	2.21	13/1/98	1.66	1.79
3/2/98	1.69	1.81	10/2/98	1.64	1.88
24/2/98	2.24	2.73	3/3/98	2.17	2.62
23/3/98	2.20	2.22	31/3/98	2.26	2.45
15/4/98	3.19	2.83			
4/5/98	2.96	2.80			

Appendix 7.5 *In vitro* organic matter digestibility (%) of Aries HD and Yatsyn 1 pastures in Experiment 4 (Chapter 7) from herbage cut samples to grazing height after grazing.

Leaders			Followers		
Date	Aries HD	Yatsyn 1	Date	Aries HD	Yatsyn 1
16/12/97	65.0	65.4	23/12/97	57.7	57.7
13/1/98	78.8	68.7	19/1/98	57.8	64.9
10/2/98	72.6	70.7	17/2/98	61.0	55.2
3/3/98	58.8	59.3	10/3/98	63.2	60.9
31/3/98	58.4	56.2	7/4/98	61.7	62.0

Appendix 8.1 Mean (20-year) monthly rainfall, soil temperature (10 cm), air temperature and maximum air temperature at AgResearch Palmerston North.

Month	Monthly mean rainfall (mm)	Monthly mean soil temperature (°C)	Monthly mean air temperature (°C)	Monthly mean maximum air temperature (°C)
September	82.2	9.8	10.5	14.9
October	86.2	12.4	12.6	16.6
November	81.9	14.6	14.3	18.4
December	89.9	16.7	16.3	20.6
January	63.3	17.9	17.8	22.3
February	71.3	17.8	18.1	22.7
March	81.3	15.9	16.4	20.8
April	79.8	13.1	14.0	18.3