ENDOPHYTIC PERENNIAL RYEGRASS AND REPRODUCTIVE PERFORMANCE OF THE EWE

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy
in Animal Science
at Massey University

Richard Hart Watson

2000

ABSTRACT

Watson, R.H. 2000. *Endophytic perennial ryegrass and reproductive performance of the ewe.* PhD thesis, Massey University, Palmerston North, New Zealand, 224pp.

A series of grazing and indoor trials were conducted to investigate the effects of perennial ryegrass infected with *Neotyphodium lolii* and its toxins on the reproductive performance of the ewe. Comparisons made were in ovulation rate, conception rate, lambs carried at scanning and lambs born per ewe, milk production and lamb growth rate between groups of ewes grazing either endophyte-infected (E+) or endophyte-free (E-) perennial ryegrass. Differences in ewe liveweight and its relationship with feed intake were also determined. More intensive trials were conducted to examine the effects of endophyte toxins on the endocrine systems that regulate the oestrous cycle and lactation.

In a 4-year grazing trial in the Manawatu there were no significant differences in ovulation rate, conception rate, scanning % or lambing % between the E+ and E- groups in any year of the trial. Mean mating date was 1.8 days later ($P<0.05$) in the E+ group than in the E- group. There was a greater ($P<0.05$) proportion of non-pregnant (dry) ewes in the E+ group compared with the E- group (14% vs 6% respectively) over the entire trial period (1996-1999), but no significant difference in any of the other parameters.

Ewes in the E+ group had lower liveweights ($P<0.001$) than ewes in the E- group throughout the duration of the trial. Ewe liveweight was a significant ($P<0.05$) source of variation in ovulation rate in the E+ group in 1998. The growth rate of single and twin lambs between birth and nine weeks of age was lower ($P<0.01$) in the E+ group than the E- group in 1998 only.

Cumulative milksolid yields were lower ($P<0.05$) in the E+ single- and twin-rearing ewes compared to the E- single and twin-rearing ewes in 1997.

E+ ewes had more faecal soiling (dags) ($P<0.05$), lower serum prolactin at mating ($P<0.05$), had higher rectal temperatures during summer ($P<0.05$) than E- ewes, and left greater postgrazing dry matter residues in autumn ($P<0.05$).

In two grazing trials in Northland (1998 and 1999), there were no significant differences in ovulation rate, conception rate or the number of lambs carried per ewe at scanning between ewes grazing E+ ryegrass and ewes grazing E- ryegrass. Serum prolactin was significantly ($P<0.05$) lower in E+ ewes than in E- ewes in 1999 but was not different between the two groups in 1998.

E+ ewes lost significantly ($P<0.05$) less weight than the E- ewes prior to mating in the1998 trial, which was due to the poor establishment of the new E- pasture and hence low dry matter production. However, E+ ewes lost significantly ($P<0.05$) more weight than E- ewes prior to, and during mating, in the 1999 trial.
Reproductive results obtained in these trials were confounded by inadequate control over experimental conditions.

In a further trial in Northland, twin-bearing/rearing ewes grazing E+ ryegrass pasture were lighter than E- ewes prior to, and during lactation. Lambs reared by the E+ ewes had ($P<0.05$) lower liveweight gains between docking and weaning, and lower weaning weights than lambs reared by the E- ewes.

A trial was conducted where daily blood samples were collected for approximately one oestrous cycle (21 days) from synchronised ewes (n=20) grazing either E+ or E- ryegrass to measure levels of serum prolactin (PRL), luteinizing hormone (LH) and progesterone. Serum PRL levels were ($P<0.01$) lower in the E+ ewes than in the E- ewes during the oestrous cycle, and the pre-ovulatory PRL surge was completely abolished in the E+ ewes. Serum LH levels were not different between the E+ and E- ewes, however, this may have been due to the sampling regime not being able to eliminate the effects of diurnal variation in LH secretion. The profile of serum progesterone secretion was significantly ($P<0.05$) different between the E+ and E- ewes.

Two trials were conducted to examine the effects of the endophyte toxin, ergovaline, and ambient temperature on the major hormones regulating parturition and mammogenesis in pregnant ewes. Serum PRL levels were ($P<0.0001$) lower in ewes fed diets containing ergovaline (Ev+) than in ewes fed ergovaline-free (Ev-) diets at high ($30^{\circ}C$) ambient temperature. Serum progesterone levels were ($P<0.001$) lower in Ev+ than in Ev- ewes at both high and low ($18^{\circ}C$) ambient temperature. There were no differences in serum cortisol levels

between any group of ewes. Serum insulin levels were ($P<0.01$) lower in the Ev+ ewes than in the Ev- ewes in the low ambient temperature treatment but were not different between the groups of ewes at high ambient temperature. High ambient temperature ($P<0.1$) reduced serum insulin levels in the Ev- group.

High ambient temperature significantly ($P<0.001$) increased PRL and depressed progesterone levels in both Ev+ and Ev- ewes, and significantly ($P<0.1$) depressed insulin in the Ev- group only. There was a significant ($P<0.001$) Ev X temperature interaction for PRL and progesterone.

Mammogenesis and lactogenesis were completely abolished in the Ev+ ewes at high ambient temperature but there appeared to be no difference between the Ev+ or Ev- ewes at low ambient temperature. Ev+ ewes at both high and low ambient temperature exhibited abnormally hostile behaviour towards their offspring that was not observed in the Ev- groups.

Rectal temperatures were higher (significant, $P<0.001$) in the Ev+ ewes than in the Ev- ewes in Trial 1and in the high temperature treatment in Trial 2.

A grazing trial in which slow release chromium oxide tracer was administered to ewes and lambs grazing either E+ or E- ryegrass pasture showed that feed intakes were significantly lower in the ewes and lambs grazing E+ ryegrass compared with ewes and lambs grazing E- ryegrass. Differences in feed intake between the E+ and E- groups were related to liveweight and liveweight change.

There were also differences in grazing behaviour between the E+ and E- groups. Ryegrass components of the pasture, and especially the leaf-sheath, were less acceptable to ewes and lambs grazing E+ than E- pastures.

It is concluded that the toxins produced by the endophyte, *N. lolii*, commonly found in perennial ryegrass, have the potential to reduce fertility, milk production, lamb growth rate and the liveweight of ewes. The range of toxin levels normally associated with E+ ryegrass pastures appears to be too low to cause large negative effects on reproductive performance. However, some small effects were observed such as a delay in mating, a reduction in milk production, and a higher incidence of dry ewes in ewes grazing E+ ryegrass pasture.

Ewes and lambs grazing E+ ryegrass pasture have lower liveweight gains, which is associated with lower voluntary feed intakes. Chronically lower liveweight in ewes associated with grazing E+ ryegrass is likely to reduce life-time performance of the ewes.

ACKNOWLEDGMENTS

First and foremost I would like to thank my supervisors, Associate Professor Maurice McDonald (Massey University) and Mr Reg Keogh (AgResearch Grasslands) who have shown immeasurable patience and guidance during the last six years. I believe that I will be a better scientist for having been associated with you both.

I would also like to thank Professor Hugh Blair (Massey University) for agreeing to step-in as my chief supervisor with the retirement of Maurie. I am grateful for all your efforts.

A huge thanks must go to Syd Easton (AgResearch Grasslands) for his supervision and guidance, and to all the members of the endophyte programme at AgResearch who have enriched my learning experience immensely.

I am extremely grateful to Michelle Kirk, Margaret Mason, and Paul and Cheryl Doyle, who have, without complaint, provided technical assistance with all the experimental trials, and shown patience beyond the call of duty with tempestuous sheep and demanding PhD students.

Thanks go to Derek Sagar and Angus Petersen (AgResearch Aorangi) for accommodating the trials at Aorangi and some of the associated impracticalities of conducting research on a farm.

My sincere gratitude to Kevin and Gill Adshead for allowing trials to be conducted on their property and for showing me very generous hospitality. I would also like to thank Stephen Dill for his enthusiasm and help in animal handling, and David Baigent (Wellsford Vet Club) for performing laparoscopic examination of the Northland ewes.

My thanks to Phil Pearce (Massey University) and Jane Candy (Massey University) for their expertise in the many hormone assays performed. I would also like to thank Barry Parlane (APU, Massey University) for setting up the indoor trials.

Special thanks to Brian Tapper, Geoff Lane and Liz Davies (all AgResearch Grasslands) for alkaloid analyses of the herbage samples, and Willhelmina Martin for chromium analysis of faeces.

Finally, and most importantly, I would like to give my eternal gratitude to my mother, Lorna, my father, Brian, the rest of my family, my partner Belinda, and all my friends for all the loyal support and advice, without which I would have surely failed. This work is dedicated to you all.

TABLE OF CONTENTS

LIST OF TABLES

TABLE **PAGE**

TABLE **PAGE**

TABLE **PAGE**

TABLE **PAGE**

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

FIGURE **PAGE**

LIST OF PLATES

LIST OF ABBREVIATIONS AND DEFINITIONS

$\alpha_{(1,2)}$	adrenoreceptor
ADF	acid detergent fibre
ADG	average daily gain
ANOVA	analysis of variance
AOR	Aorangi lowland research station
BSA	bovine serum albumin
CB154	2-Br-α-ergocriptine (bromocriptine)
CHO	soluble carbohydrate
CIDR	controlled internal drug release (device)
CNS	central nervous system
CV	co-efficient of variation
cv	cultivar
°C	degrees Celsius
$D_{(1,2)}$	dopamine receptor
d	day(s)
DAR	donkey anti-rabbit antibody
DASP	double antibody/solid phase
DM	dry matter
2-4,5D	2,4-trichlorophenoxyacetic acid (herbicide)
E+	endophyte-infected
E-	endophyte-free
EA	ergopeptine alkaloids
Ev	ergovaline
g	grams; force due to gravity
GABA	gamma amino butyric acid
GGT	gamma glutamyl transpeptidase
GI	gastrointestinal
GLM	generalised linear model
GnRH	gonadotropin releasing hormone
h	head (1 animal)
ha	hectare
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptophan (serotonin)
iu	international units
K	potassium
kg	kilograms
l	litre(s)
LAA	lysergic acid amide
LH	luteinizing hormone
LOL	lolitrems
LSD	lysergic acid diethylamide
LW	liveweight
LWΔ	liveweight change
M	molar
m	metre(s)
MCPA	4-chloro-2-methylphenoxy acetic acid (herbicide)
MCPB	4-(4-chloro-2-methylphenoxy)butanoic acid) (herbicide)
ME	metabolisable energy

mg	milligram(s)
µg	microgram(s)
min	minute(s)
MJ	megajoule(s)
ml	millilitre(s)
µl	microlitre(s)
mM	millimole(s)
mm	millimetre(s)
µm	micrometre(s)
N	nitrogen
n	number
NDF	neutral detergent fibre
NIADKK	National Institute of Diabetes and Digestive and Kidney Diseases
nm	nanometre(s)
NRS	normal rabbit serum
NSB	non-specific binding
oLH	ovine luteinizing hormone
OMD	organic matter digestibility
oPRL	ovine prolactin
P	phosphorus
P(<)	probability
pg	picograms
PMSG	pregnant mare serum gonadotropin
ppb	parts per billion
ppm	parts per million
RGLB	ryegrass leaf blade
RGLS	ryegrass leaf sheath
RGS	ryegrass staggers
RIA	radio-immunoassay
rpm	revolutions per minute
SEM	standard error of the mean
v/v	volume per volume
WT	weight
↑	increase
↓	decrease

'wild-type'	naturally occurring endophyte in perennial ryegrass.
Fertility	used to collectively describe ovulation rate, conception rate, spermatogenesis in the ram, and mating behaviour.
Reproductive performance	encompasses all aspects of ewe reproduction including milk production, and growth rate and health of offspring.
Reproductive rate	number of offspring born per female.

CHAPTER I

General introduction

The fungal endophyte (*Neotyphodium lolii* Latch, Christensen & Samuels) exists in a symptomless and mutualistic association within perennial ryegrass (*Lolium perenne* L.).

The presence of a fungal endophyte in perennial ryegrass was reported in New Zealand by Neill (1941). Researchers at the time concluded that there were no adverse effects of the endophyte presence on animal performance (Cunningham, 1958).

It was not until forty years later that the significance of endophyte presence in pasture to animal production was discovered.

The association of the endophyte *Neotyphodium coenophialum* in tall fescue with a specific range of animal health problems was first noted by Bacon *et al.* (1977).

It has since been verified in several reports that *N. coenophialum* is responsible for the range of symptoms that are collectively referred to as fescue toxicosis. These symptoms include reduced liveweight gains, increased body temperature, rough hair coat, gangrenous necrosis of tissue in feet, tail and ears, reduced milk production and reproductive performance (Thompson & Stuedemann, 1993).

Not long after the discovery by Bacon, an association was noted between the presence of *N. lolii* in perennial ryegrass and ryegrass staggers in sheep (Fletcher & Harvey, 1981). Ryegrass staggers is a neuromuscular disorder that affects several species of domestic animal grazing ryegrass pastures in the summer and autumn (Gallagher *et al.*, 1977). The endophyte toxin, lolitrem B, produced by *N. lolii* in perennial ryegrass has since been identified as the major causative agent of ryegrass staggers (Blythe *et al.*, 1993). Staggers are not exhibited in animals grazing E+ tall fescue because *N. coenophialum* does not produce lolitrem B or other neurotoxins.

Ergovaline, which is the principle ergopeptine alkaloid implicated in E+ fescue toxicosis, has also been detected in E+ ryegrass pastures, although at lower levels (Rowan & Shaw, 1987).

It is, therefore, possible that many of the animal health problems observed in animals grazing E+ tall fescue will also occur in animals grazing E+ ryegrass. Closer examination of animals grazing E+ perennial ryegrass has identified similarities to many of the toxicoses observed in animals grazing E+ tall fescue. These similarities include reductions in weight gain, depressed serum prolactin and increased body temperature in sheep (Fletcher *et al.,* 1999). Lower milk production has been observed in cows grazing E+ ryegrass pastures compared with E- pastures (Blackwell & Keogh, 1999). Cattle in Northland, New Zealand, grazing E+ ryegrass pastures also frequently suffer from heat stress in the summer months in much the same way as cattle grazing E+ tall fescue (Easton *et al.,* 1996).

The fact that perennial ryegrass exists in nature in an endophyte-free state would appear to offer a solution to the animal health problems. However, shortly after it was shown that endophyte was responsible for these animal disorders, Gaynor *et al.* (1985) found that the endophyte alkaloid, peramine, offered protection to the ryegrass plant against attack from argentine stem weevil (*Listronotis bonariensis*). Subsequent research has shown that peramine and other endophyte toxins (Prestidge and Ball, 1996) may also adversely affect several other species of insect pest.

It is, therefore, considered necessary for endophyte-infected ryegrass seed to be sown to ensure the persistence of pastures under many of New Zealand's environmental conditions and insect pest pressures encountered in them. This requirement for endophyte has resulted in the widespread use of endophyte-infected ryegrass throughout New Zealand.

Poor reproductive performance is a major problem in horses, cattle and sheep grazing E+ tall fescue pastures (Porter & Thompson, 1992). This poor reproductive performance is generally characterised by lower numbers of offspring born per female mated (reproductive rate), reduced milk production and lower growth rate of offspring. Given the reported presence of some similarities between E+ tall fescue and E+ ryegrass in animal toxicosis, it is possible that E+ ryegrass may also affect reproductive performance in similar ways to E+ tall fescue. The effects of grazing E+ ryegrass on the reproductive performance of grazing animals are yet to be fully investigated. In the case of sheep, even reports on the effects of E+ tall fescue on reproductive performance are sparse.

Reports prior to the present study had not observed significant differences in the number of lambs born between ewes grazing E+ and E- ryegrass pasture (Eerens *et al.,* 1994; L.R. Fletcher, personal communication). However, these trials were not specifically designed to examine reproductive performance, and the study by Eerens *et al.* (1994) was conducted in a cool, moist environment where the adverse effects on animal performance may be reduced.

The reproductive performance of New Zealand ewe flocks is considered to be well below their potential (Knight, 1990). Given that the majority of these flocks are grazing E+ ryegrass-dominant pastures it is possible that at least some of this reduction in performance may be due to endophyte toxins.

Despite the growing evidence that reproductive performance may be reduced in animals grazing E+ pasture, many of the underlying mechanisms by which endophyte toxins cause these reductions remain unclear. Many of the toxins produced by endophytes have been found to have a wide range of physiological activity (Oliver, 1997) and much of this activity is associated with functions that directly affect the reproductive system. These activities may include important factors such as hormone regulation of the oestrous cycle, parturition and lactation.

Poor ewe and lamb liveweight gains associated with grazing E+ tall fescue and E+ ryegrass pastures may have adverse effects on reproductive performance and should, therefore, be part of any investigation on the effects of E+ ryegrass on reproductive performance.

The importance of the sheep industry to New Zealand, and the current reliance on E+ perennial ryegrass, make it vital to examine any possible losses in productivity due to the presence of endophyte and endophyte toxins. A major contributing factor to production losses due to grazing E+ ryegrass, may be poor reproductive performance of ewes grazing these endophyte-infected pastures. It is important that the size and nature of the effects of E+ ryegrass on all aspects of sheep reproduction be determined so that ways to effectively treat and/or prevent any such problems can be developed and implemented.

CHAPTER II

Review of literature

1. Introduction

The few reports on the effects of endophyte on reproductive performance in grazing animals have mostly been associated with tall fescue (*Festuca arundinacea* Schreb.) pastures in North America infected with the endophyte fungus *Neotyphodium coenophialum.*

Reduced reproductive efficiency and weight gains (Hoveland, 1992) are common consequences of grazing *N. coenophialum*-infected tall fescue. The effects of endophyte-infected (E+) tall fescue on reproductive performance have been examined in many species of grazing livestock and in laboratory animals and results have varied greatly between species and the ways in which the effects are manifested.

This review covers the currently published reports on the effects of grazing endophyte-infected pasture or endophyte toxins on the reproductive performance of the major species of domestic grazing animals and laboratory animals. Almost all of these reports are associated with E+ tall fescue. However, the similarities in toxin production with E+ perennial ryegrass (Rowan *et al.,* 1990) make these reports relevant to the present study. The established endocrinological and physiological effects of the major groups of endophyte toxins associated with animal toxicoses are also reported with reference to the possible direct or indirect implications to reproductive performance. The review also includes the effects of E+ pasture on milk production and liveweight gain as integral components of reproductive performance. Finally, production of the major toxins involved in animal toxicosis by E+ perennial ryegrass is reviewed.

2. Effects of endophyte on equine fertility

The effects of E+ tall fescue on reproduction in the horse are particularly severe and are well documented.

Reproductive dysfunction in horses grazing tall fescue was reported before the endophyte was implicated as the cause. A survey in Missouri, involving 298 horse farms and 1010 mares, reported that 26.8% of the farms with tall fescue had reproductive problems with mares compared to only 11.5% of farms using other forage systems (Garrett *et al.,* 1980). This survey indicated that 53% of mares grazing tall fescue pastures were agalactic, 38% had prolonged gestation, and 18% lost foals because of prenatal mortality. In 1981, Harper & Henton reported information provided by some horse owners that suggested horses grazing tall fescue frequently had reproductive problems. The most common reproductive disorders observed were agalactia, thickened placentas, spontaneous abortion, dead or weak foals at birth, and rebreeding problems. This same study reported that 27% of mares grazing tall fescue pastures had some type of reproductive problem compared with reproductive problems in only 9% of mares grazing pastures without fescue. Poppenga *et al.* (1984) described mare agalactia, placental thickening, and high foal mortality associated with E+ tall fescue and Taylor *et al.* (1985) reported similar reproductive abnormalities. A study of horse farms in Kentucky indicated that as many as 40% of mares grazing E+ tall fescue had reproductive abnormalities (Barnett, 1985).

When the endophyte (*N. coenophialum*) in tall fescue was shown to be associated with summer toxicosis in cattle (Hoveland *et al.,* 1980; Hoveland *et al.,* 1983), it was assumed the fungal endophyte was the putative agent in horses. Since a role for endophyte in these reproductive problems was indicated, studies have been conducted examining the effects of the endophyte on reproductive performance in the horse.

Putnam *et al.* (1991) examined the effects of fungal endophyte in tall fescue on pregnant mares and foetal viability. This study found that ten out of eleven mares grazing E+ tall fescue had obvious dystocia compared with no mares with dystocia in the endophyte-free (E-) tall fescue treatment, and foal viability was severely reduced (Table 2.1.). Foals born to mares grazing the endophyte-infected grass were described in this study as dysmature, with overgrown hooves, poor and irregular incisor eruption, long hair coats and large and poorly muscled skeletal frames. Placental abnormalities included oedema, fibrosis, and mucoid degeneration.

Table 2.1. Effects of consuming tall fescue infected with endophyte fungi on pregnant mares and their foals (Putnam *et al.*, 1991)

	E- ***(n = 11)***	***E+*** ***(n = 11)***
Foals carried to term	11	11
Foals alive at birth	11	3
Foals surviving natal period	11	1
Number of mares with dystocia	0	10
Mares surviving	11	7
Mares lactating	11	1

The mean duration of gestation was significantly greater for animals in the E+ treatment, being on average 20 days longer than in the E- treatment. This study strongly implicated the presence of endophyte as the causative agent of reproductive problems and perinatal foal mortality in pregnant mares grazing tall fescue.

All these studies indicate that the major effects of the endophyte on the horse are associated with pregnancy and the subsequent lactation. It is believed that many of the effects of endophyte on horse reproduction are caused by ergopeptine alkaloids produced by the fungus.

Very little information exists relative to the effects of endophyte on the reproductive system of stallions. It is possible that maintaining stallions on E+ pasture during the breeding season could reduce semen volume (Cross, 1997).

3. Effects of endophyte on bovine fertility

The effects of grazing E+ perennial ryegrass and tall fescue pastures have also been examined in cattle. However, this has been done almost exclusively with cattle grazing E+ tall fescue. Reduced reproductive efficiency in cattle grazing E+ tall fescue is part of a syndrome often referred to as fescue summer toxicosis.

Cows grazing E+ tall fescue have had significantly reduced calving rates compared to cows on E- tall fescue. Boling (1985) found that calving rates for cows grazing E+ tall fescue were 67%, compared with 86% for cows grazing E- tall fescue. Schmidt *et al.* (1986) reported pregnancy rates of 96% for cows grazing E- but only 55% for cows grazing E+. This study also found that conception rates were correlated with the level of endophyte infection with a 3.5% decrease in conception rate for every 10% increase in the level of endophyte infection. Washburn *et al.* (1989) found that conception rate with artificial insemination was reduced at the first service in heifers grazing E+ forage compared with heifers grazed on E- forage (45 vs 75%). Several other reports have found that cows grazing E+ tall fescue have had significantly lower calving rates (Gay *et al.,* 1988; Essig *et al.,* 1989; Tucker *et al.,* 1989; McDonald, 1989).

Grazing E+ forage has been shown to affect the onset of puberty in cattle. Puberty was delayed in Angus heifers raised on E+ fescue (Washburn *et al.,* 1989, 1991). The first sustained increase of serum progesterone was used to determine puberty and these values indicated that 0 vs 32% (1^{st} year), 26 vs 53% (2^{nd} year) and 5 vs 26% (3^{rd} year) of heifers reached puberty at 15 months in the E+ and E- treatments respectively.

The effects of endophyte on ovarian gamete maturation, ovulation, gamete transport, and embryo attachment are unknown (Porter & Thompson, 1992).

The effects of endophyte on reproduction have also been investigated for bulls. Results from a trial feeding E+ tall fescue hay to Holstein bulls indicated that the presence of endophyte in forage did not appear to have severe detrimental effects on body growth or reproductive development (Evans *et al.,* 1988). However, Alamer & Erickson (1990) reported that the endophyte reduced GnRH-stimulated testosterone secretion in 3 month old bulls. Bass *et al.* (1977) found that bulls grazing E+ perennial ryegrass had lower serum testosterone levels that coincided with severe ryegrass staggers. It is, therefore, possible that endophyte-infected grass may impair testicular function.

4. Effects of endophyte on laboratory animal fertility

The effects of endophyte have been examined in various species of laboratory animal.

4.1. Rats

A trial by Daniels *et al.* (1981) in which female rats were fed a toxic tall fescue extract for 42 days starting at the 7th day of pregnancy, resulted in only two of the seven rats giving birth to live young. One of these litters contained only five pups, with one being still born. Three of the seven rats aborted, one gave birth to seven stillborn pups and one female died during birth. All female rats fed the non-toxic diet gave birth to normal litters. Female rats fed diets containing tall fescue seed with 40% endophyte infection had decreased mean body weight of uteri, failed to maintain normal oestrous cycles, which were in most cases either extended or stopped completely, and did not become pregnant (Varney *et al.*, 1987). This study found no alteration in conception rate, however, there was a reduction in number of embryo implantations in the rats fed the toxic seed.

Endophyte has also been shown to affect fertility in male rats. Male rats fed tall fescue seed infected by an endophyte fungus had retarded gonadal and epididymal development, depressed sperm production ability, and lower testicular weights (Zavos *et al.*, 1986).

4.2. Mice

The presence of endophyte in the diet has been shown to cause reproductive dysfunction in mice. Male and female CD1 mice fed diets containing different amounts of E+ (80% infection) tall fescue seed showed increased negative effects on the number of pups born per litter and weight of the pups as the proportion of E+ seed in the diet increased (Zavos *et al.*, 1987a; 1987b). This study showed that the reproductive capacity of both male and female was affected. The effects of feeding E+ tall fescue seed to mice dams during gestation and lactation, on the subsequent growth and sexual maturity of their male and female offspring has been examined (Varney *et al.*, 1991a). This study showed that female pups born to dams fed diets containing the infected tall fescue seed weighed significantly less that those born to the control dams. Varney *et al.* (1991b) showed that the congenital effects on mice pups born of

dams fed E+ diet was significant in lowering the growth rate of the pups during suckling. The dam's ability to provide nourishment to pups was also lowered.

These studies conducted with mice have found that females appear to be more prone to reproductive dysfunction than males when consuming diets containing E+ tall fescue.

4.3. Rabbits

Daniels *et al.* (1984) examined the physiological responses in pregnant white rabbits fed an extract of E+ tall fescue. This study showed that rabbits given the fescue extract lost body weight rapidly, had elevated body temperature and respiration rate. In addition, does given the toxic extract aborted or gave birth to stillborn pups. Pups born live died within two days due to lack of milk production by the doe. Additionally, Cottam *et al.* (1997) found that kit mortality was increased in feral rabbits offered diets containing the ergot alkaloid bromocriptine (CB154).

5. Effects of endophyte on deer fertility

The specific effects of grazing E+ pastures on deer have not been examined, however, deer grazing E+ perennial ryegrass are often severely affected by the neuromuscular disorder referred to as ryegrass staggers (RGS) which is caused by toxins produced by the endophyte (Orr & Mackintosh, 1985). RGS has been known to cause death in both adult deer and fawns (Mitchell & McCaughan, 1992). Other symptoms identified in deer grazing E+ pastures are increased respiration rate and body temperature (Mackintosh *et al.*, 1982).

Stevens *et al.* (1992) found that there were no differences in productivity between deer grazing E+ or E- perennial ryegrass. However, there were differences in behaviour with the deer on the E+ treatment being more flighty, harder to handle and keep behind an electric fence than the deer on the E- treatment.

6. Effects of endophyte on ovine fertility

There are few reports on effects of endophyte on sheep fertility and there are contradictions as to these effects and their severity.

It was observed that ewes grazing E+ tall fescue have delayed conception after introduction of the ram (Bond *et al.,* 1988). However, this study reported no effects on body weight gains, gestation length, average number of lambs born, lamb birth weight, or lamb survival.

Eerens *et al.* (1998) found that ewes grazing E+ ryegrass in a cool moist environment had a 4-day delay in parturition date with no differences in lamb birth weight. This delay has been attributed to embryonic mortality and to a delay in the onset of oestrus rather than to fertilisation failure or extended gestation length (Bond *et al.,* 1982; 1988).

Recently it was shown that ewes grazing E+ tall fescue that contained the endophyte toxin ergovaline during mating had lower ovulation rates and number of lambs carried to 90 days of gestation than ewes grazing E+ tall fescue that was ergovaline-free (Kramer *et al.,* 1999)

7. Mechanisms by which endophyte reduces fertility

7.1. Pharmacological and physiological effects of endophyte toxins

This review only deals with groups of endophyte toxins implicated in mammalian toxicosis.

Very little is known about the underlying mechanisms involved in the reduction of reproductive performance in animals grazing E+ pastures. Identifying the toxins involved and their physiological role has proven difficult owing to the presence of several variant compounds within the major toxin groups, each with a wide range of physiological activity.

There have been reports on some on the physiological activities of the different endophyte toxins and there is increased ability to differentiate their effects and potential physiological role.

The four main groups of toxins produced by the endophyte fungus, implicated in animal toxicosis, are ergopeptine alkaloids, lysergic acid amides, lolines and the lolitrems, which are of concern in E+ ryegrass.

Some of the physiological effects of the major endophyte toxins are summarised in Table 2.2.

7.1.1. Ergopeptine alkaloids

Ergopeptine alkaloids, of which ergovaline is the most abundant in E+ forage, have been found to have several pharmacological effects in mammals. The ergopeptine alkaloids bind at D_1 and D_2 dopamine receptors (Rhodes *et al.,* 1989). Pharmacological activity of the ergot alkaloid group of endophyte toxins is different for D_1 and D_2 receptors. These alkaloids interact with the D_2 receptor in an agonistic fashion, whereas interaction with the D_1 receptor is in an antagonistic fashion (Siegel *et al.,* 1989). D_1 receptors control vasodilation and parathyroid hormone release and D_2 receptors inhibit norepinephrin release, depress chemosensory activity and depress prolactin and α-melanocyte-stimulating hormone (Cooper *et al.,* 1991). D_3, D_4, and D_5 dopamine receptors are also present in the body. However, the relationship of ergopeptine alkaloids with these receptors is not known at present.

Badia *et al.* (1988) found that the ergopeptine alkaloid, ergotamine, is an agonist at both α-1 and α-2 adrenergic receptors, and also can be an antagonist at α-1 receptors. α-adrenergic receptors mediate coronary and skeletal muscle contractions, pulmonary, visceral and renal vasoconstriction, increase stomach and intestinal motility and tone, have stimulatory roles in male and female sex organs, and various secretory functions throughout the body such as decreasing insulin and glucagon secretion and increasing growth hormone secretion. α-2 receptors prevent further release of norepinephrin, modulate the function of other neurones including those using serotonin, dopamine and acetylcholine, reduce the turnover rate of these nerves after stimulation, and are involved with smooth muscle contraction (Daunt & Maze, 1992).

There is evidence that ergopeptine alkaloids bind at serotonin-2-receptors (5-HT_2). Dyer (1993) found that ergovaline had agonistic effects on $5HT_2$-receptors. $5HT_2$-receptors are involved in contraction of both arteries and veins in rats (Cohen *et al.,* 1993). Serotonin is

known to enhance the effect of angiotensin II (Doyle, 1988). Serotonin receptors have important effects on gastrointestinal motility. Sheep administered serotonin had a sustained increase in gastrointestinal muscle tone, with inhibition of extrinsic reticulo-rumen contractions (Ruckebusch & Ooms, 1983). Serotonin is found in relatively high concentration in the brain, and there is evidence that it may play an excitatory role in the regulation of prolactin secretion, inhibit transmission in pain pathways in the dorsal horns and may be involved in the regulation of circadian rhythms (Ganong, 1989).

7.1.2. Lysergic acid amides

Lysergic acid amides have been shown to have some antagonist activity at α1-adrenergic receptors, however, they appear to have a higher affinity for $5HT_2$ receptors (Oliver *et al.*, 1993a).

Lysergic acid amides differ from ergopeptine alkaloids in their affinity for the various receptors discussed. Krisch *et al.* (1992) observed that the larger ergopeptine alkaloids have higher affinity for α-adrenoreceptors, as opposed to 5-HT or dopamine receptors, which was opposite to that found with the smaller lysergic acid amides.

Lysergic acid diethylamide (LSD) binds to $5\text{-}HT_2$ receptors in the brain which results in hallucinations and other mental aberrations in humans (Ganong, 1989) and it is speculated that serotonin has a role in regulating animal behaviour and sleep (Whitaker-Azmitia & Peroutka, 1990).

7.1.3. Lolines

Lolines have been found to have antagonist activity at α-2 adrenoreceptors (Oliver *et al.*, 1990) and agonist activity at $5HT_2$ receptor sites (Oliver *et al.*, 1993b)

7.1.4. Lolitrems

Lolitrems cause a neuromuscular response in livestock that is commonly referred to as ryegrass staggers (RGS) (Gallagher *et al.*, 1977). This disorder (RGS) is characterised by tremors and locomotor inco-ordination (Cunningham & Hartley, 1959).
These toxins cause tremors in skeletal muscle and have inhibitory effects on the smooth muscle of the gut (Smith *et al.*, 1997).

Sheep showing severe symptoms of RGS have deranged release of the excitatory amino acid neurotransmitters aspartic acid and glutamic acid in the cerebrocortical synaptosomes (Mantle, 1983). Munday-Finch & Garthwaite (1999) have found that lolitrems distribute randomly throughout the fatty brain matrix, which masks the binding to specific sites. It was found in mice injected with 14C-labled tremorgen that only a very small amount of tremorgen reached the brain and spinal cord, which indicates that the receptors involved are extremely sensitive to these compounds.

7.1.5. Alkaloid synergism

There are very few reports on any possible synergistic reactions between the alkaloids. The possibility of synergism between fungal toxins was suggested by Gallagher *et al.* (1977), who investigated the effects of fungal tremorgens, similar to those produced by endophyte, on sheep. Fletcher & Easton (1997) reported that lolitrem B and ergovaline may synergistically interact to increase the severity of RGS. However, no synergistic properties were exhibited between ergotamine and N-acetyl loline (Oliver *et al.*, 1990).

7.2. Dopaminergic effects of endophyte toxins

The ergopeptine alkaloid group of endophyte toxins have dopaminergic activity (see section 7.1.1). It is therefore possible that these toxins may affect animal reproduction as dopamine agonists.

The administration of dopamine has inhibited the luteinizing hormone (LH) response to a bolus of GnRH in sheep (Deaver & Daily, 1982; Donnelly & Dailey, 1991), rabbits (Daily *et*

al., 1978) and humans (Judd *et al.,* 1978). Dopamine receptors are present on a sub-population of gonadotrophs (Goldsmith *et al.,* 1979) and it is possible that dopamine acts directly on the gonadotroph to decrease the binding of GnRH. Dopamine has also been shown to reduce basal progesterone release from bovine corpora lutea *in vitro* (Rhodes & Randel, 1982).

In addition to direct actions of dopamine on the reproductive system there may be indirect effects mediated through the depression of prolactin (see section 7.5.).

7.3. Serotonergic (5HT) effects of endophyte toxins

Ergopeptine alkaloids have serotonergic activity (Dyer, 1993; Oliver *et al.,* 1993b). Cattle grazing E+ tall fescue appear to have more reactive 5-HT_2 receptors than cattle on E- fescue (Oliver *et al.,* 1996).

Serotonin receptors are present throughout the CNS, in blood vessels, in the gastrointestinal tract and on platelets (Martin, 1994), and serotonin has regulatory roles in the mammalian reproductive system.

Ewes given an increasing dose of serotonin showed a linear increase in plasma LH (Deaver & Daily, 1983). Deaver & Daily (1982) found that serotonin potentiated the release of LH following treatment with GnRH, and Philo & Reiter (1980) demonstrated that serotonin peaks correspond with a lower fertility in cattle during the winter months.

In the female rat, serotonin has a central role in the control of the preovulatory LH surge and subsequent ovulation (Coen *et al.,* 1980). In male rats serotonin administration (10 mg/kg body weight) can detrimentally affect spermatogenesis but does not affect leydig cell function (Gonzales *et al.,* 1981).

Endophyte toxins may affect early pregnancy due to serotonergic activity. Dyer (1993) found that ergovaline is a potent vasoconstrictor of isolated uterine and umbilical arteries and that this is mediated via serotonin receptors.

Serotonin, administered on the day after the initiation of embryo implantation, promptly terminates pregnancy in the rat (Lindsay *et al.*,1963). The disruption of implantation after serotonin administration is not a result of impaired corpus luteum function, but is associated with marked and protracted reduction in uterine blood flow and intraluminal oxygen availability which is consistent with the vasoconstriction activity of serotonin (Mitchell & Hammer, 1983).

7.4. α-adrenergic effects of endophyte toxins

Badia *et al.* (1988) found that the ergopeptine alkaloid, ergotamine, is an agonist at both α_1 and α_2 adrenergic receptors. The possible adrenergic effects of endophyte toxins on fertility have not been documented. α-receptors are present throughout the body and have several roles. α-receptors are present in the uterus and penis and control uterine motility and penile erection (Ganong, 1989).

α-adrenergic receptors are also involved in regulation of reproductive hormone secretion. Studies in female rats have shown that α_1-adrenergic densities undergo a diurnal rhythm in brain regions associated with entrainment to the photoperiod. Oestrogen alters the rhythm of these receptors in areas involved with the regulation of LH secretion, and decreases the density in other oestrogen-responsive regions (Weiland & Wise, 1987). Clifton & Sawer (1980) showed that noradrenalin, via α-adrenergic receptors, acts as a modulator of LH secretion but is not indispensable for feed-back control mechanisms.

7.5. Prolactin suppression by endophyte toxins

Grazing E+ tall fescue pastures has caused a reduction in serum prolactin in horses (Redmond *et al.*, 1994), cattle (Bond & Bolt, 1986) and sheep (Bolt *et al.*, 1982). Feeding toxic extracts from E+ tall fescue has depressed serum prolactin in rats (Porter *et al.*, 1985).

The reduction in prolactin is most likely caused by ergopeptine alkaloids produced by the endophyte fungus. The ergopeptine alkaloids are dopamine agonists (Rhodes *et al.*, 1989). Although several hormones and substances have been shown to exert direct suppressive

effects on pituitary prolactin, dopamine from the hypothalamus via the hypophysial portal system acting on the anterior pituitary lactotrophs is the main inhibitory regulator (Lamberts & MacLeod, 1990). It is, therefore, believed that the ergopeptine alkaloids depress prolactin secretion through dopaminergic and antiserotonergic activities (Berde & Schild, 1978).

Prolactin has several roles in regulating reproductive processes in animals. Nicoll & Bryant (1972) described effects of prolactin in mammals, which included synergistic effects with steroid hormones on male and female gonads and conditioning effects on male sex accessory glands. Prolactin appears to be of particular importance in control of reproduction in rodents. Prolactin regulates corpus luteum function and gonadotrophin secretion in female rats (Smith, 1980). Low prolactin levels may reduce testis steroidgenesis during puberty in the male rat (Suescun *et al.,* 1985). Chandrashekar *et al.* (1987) demonstrated that subnormal concentrations of prolactin reduce the sensitivity of the hypothalamic-pituitary system to feedback inhibition by testosterone in rats.

The effects of reduced prolactin in other species of animal is less well understood. It was concluded that prolactin has a regulatory role in steroid secretion by luteal tissue of gilts in the mid and late periods of pregnancy (Szafranska *et al.,* 1992) which may have some similarities to its function in the rat. To date there are very few reports on the effects of hypoprolactinaemia in grazing animals, however, there is evidence that the reduction in serum prolactin in animals grazing E+ grasses may be instrumental in reduced fertility.

Prolactin may play a role in stimulating the equine ovary. Nequir *et al.* (1993) found that when endogenous prolactin secretion is increased during anoestrous by dopamine receptor blockade or by administering prolactin, typical transitional follicular growth commences, so that low prolactin levels may retard this process. This, however, does not appear to be the case in cattle. Bevers *et al.* (1985) found that follicles (> 5mm) from the ovaries of cattle treated with bromocriptine did not bind sheep prolactin irrespective of their quality or diameter and, therefore, it was concluded that prolactin is not involved in the growth and development of follicles (> 5mm) in cattle.

There is increasing evidence that prolactin has important roles in the fertility of males of species other than rats. It was found that stallions treated with the ergot alkaloid bromocriptine

had decreased serum prolactin levels and reduced semen volume after sexual stimulation (Thompson *et al.,* 1996). Therefore, it is possible that reduced serum prolactin could have an effect on semen production. Rams treated with CB154 to lower serum prolactin did not show reduced numbers of LH receptors, which is in contrast to the results found in rodents. However, a delay in the beginning of testicular growth was observed in the rams treated with CB154 (Barenton & Pelletier, 1980). In an earlier study, ram lambs treated with CB154 showed no significant decrease in testis weight and the establishment of spermatogenesis was not delayed. However, there was a marked decrease in the weight of seminal vesicles and in their fructose concentration (Ravault *et al.,* 1977). A study by Evans *et al.* (1988) found that bulls grazing E+ tall fescue had significantly lower prolactin levels than bulls grazing E- tall fescue. However, there appeared to be no detrimental effects on reproductive development.

The effects of low prolactin on reproduction in the grazing animal are unclear and more studies are required to determine prolactin's role in reduced fertility in livestock grazing E+ pasture.

7.5.1. Prolactin and milk production in animals grazing E+ feed

The suppression of prolactin in animals grazing E+ pastures may also affect lactation, which is an integral part of the reproductive process.

Cattle grazing E+ tall fescue have had depressed milk yields (Hemken *et al.,* 1979; Schmidt *et al.,* 1986). Stilham *et al.* (1982) found that milk production was reduced by 59% in ewes grazing toxic tall fescue compared to ewes grazing wheat. Prolactin is involved in lactogenesis and mammogenesis in cattle but not in galactopoiesis (Karg & Schams, 1974) Therefore, decreased serum prolactin after the start of lactation does not result in depressed milk production, (Schams *et al.,* 1972).

The effects of endophyte on milk production are particularly severe in horses (Monroe *et al.,* 1988) and rabbits (Daniels *et al.,* 1984) which commonly exhibit agalactia. The greater effect on lactation in horses and rabbits is due to the lack of placental lactogen in these species and, therefore, a complete reliance on prolactin to stimulate prepartum lactogenesis (Forsyth, 1986). Cattle and sheep have both placental lactogen and prolactin (Forsyth, 1986). The

depressive effects of ergotpeptine alkaloids on prolactin secretion may suppress prolactin's effects on lactogenesis in cattle and sheep, but have no effects on placental lactogen (Cross, 1997) and therefore effects on milk production are not as severe.

7.6. Effects of endophyte toxins on luteinizing hormone (LH) secretion

The effects of endophyte on LH secretion in the grazing animal are not well understood and evidence to date has been conflicting. Mizinga *et al.* (1992) found that endophyte had no effect on LH secretion (basal, pulse frequency or amplitude) in cyclic heifers. Similarly Christopher *et al.* (1990) reported no difference in LH secretion between ovariectomized beef heifers grazing E+ or E- tall fescue.

Rams dosed with the ergot alkaloid 2-Bromo-α-ergocriptine had no significant reduction in serum LH levels (Ravault *et al.,* 1977; Barenton & Pelletier, 1980). However, Browning *et al.* (1997) found that serum LH was significantly reduced in steers given a single intravenous dose of ergonovine or ergotamine, both of which are similar to ergot alkaloids produced in E+ pastures.

Table 2.2. Some physiological effects, and possible mechanisms, of the major endophyte toxins, ergopeptine alkaloids (EA), lysergic acid amides (LAA), and lolitrems (LOL) on various organs or systems in the grazing animal (Summarised from a review by Oliver (1997)).

ORGAN/SYSTEM	EFFECT	TOXIN(S)	MECHANISM
LUNGS/RESP.	▪ ↑ Respiration rate ▪ Bronchoconstrict.	EA, LAA??, LOL??	α_2, 5HT receptor activity
GI TRACT	▪ ↑↓ Smooth muscle activity ▪ ↑ Faecal moisture	LOL, EA?, LAA??	α_2, 5HT, D_1/D_2 receptor activity?
VASCULAR	▪ Vasoconstriction ▪ Hyperthermia	EA, LAA? LOL?	α_1/α_2, $5HT_2$ receptor activity?
SKELETAL MUSCLE	▪ Tremors (RGS)	LOL, EA??, LAA??	↑ neurotransmitter activity
MAMMARY GLAND	▪ ↓ Milk production	EA, LAA??, LOL??	↓ Prolactin ↑↓ Other hormones regulating lactation?
SEX ORGANS	▪ ↓ Reproductive rates ▪ ↓ Ovulation rate	EA, LAA??, LOL??	↑↓ Hormones regulation the reproductive system?

? Is suspected

?? Is unknown

7.7. Effects of endophyte toxins on progesterone secretion

There is evidence that grazing E+ tall fescue affects progesterone production in horses and cattle. Pregnant mares grazing E+ tall fescue pasture have lower blood concentrations of progesterone (Monroe *et al.*, 1988), however, in cyclic mares it was found that serum progesterone levels were elevated on E+ pasture (Brendemuehl *et al.*, 1994b).

Mahmood *et al.* (1994) found that grazing E+ pasture disrupted serum progesterone profiles in weaner heifers and suggested the cause was a higher incidence of luteal dysfunction. Other reports have failed to find any effect of grazing E+ pasture on serum progesterone levels in cattle (Fanning *et al.*, 1992).

7.8. Effects of endophyte toxins on cortisol secretion

Foals born to mares grazing E+ tall fescue have depressed cortisol levels (Brendemuehl *et al.*, 1994a). Foetal cortisol levels are important in the initiation of parturition. Normally, foetal cortisol levels increase prior to parturition in response to adrenocorticotrophic hormone (ACTH) (Sharp & Bozar, 1995). Therefore, the depression of cortisol in the foal could be another cause of prolonged gestation.

7.9. Effects of endophyte toxins on estradiol-17β secretion

Gravid mares grazing E+ tall fescue have had elevated plasma estradiol-17β (Redmond *et al.*, 1993; Redmond *et al.*, 1994). Estrogens are important in the maintenance of pregnancy (Pashen, 1984) and mammary development (Cross *et al.*, 1995). When parturition nears, estrogens stimulate prostaglandin production and oxytocin receptor synthesis in the uterus (Vivrette, 1994).

It is hypothesised that the ergopeptine alkaloids prevent oestrogen binding to its receptors which stops negative feedback and subsequently oestrogen levels in circulation increase (Cross, 1997). Without the opportunity to bind to its receptors, estrogens might not be able to properly stimulate prostaglandin and oxytocin receptor synthesis, thereby prolonging gestation (Cross, 1997).

7.10. Effects of endophyte toxins on gamma-aminobutyric acid (GABA) secretion

Dihydrogenated ergopeptine alkaloids are known to bind to the GABA receptor-associated chlorine ionophore in the brain of mice (Tverdeic & Pericic, 1991). It is, therefore, likely that the same group of compounds produced by endophyte will also bind to GABA receptors and disrupt the associated physiological processes, including reproduction.

The effects of ergopeptine alkaloids, produced in E+ perennial ryegrass and tall fescue pastures, on GABA activity in the grazing animal have not been investigated. However, it is possible that fertility may be affected via ergopeptine alkaloids binding to GABA receptors and studies are required to investigate this possibility.

8. Indirect effects of endophyte toxicosis on reproduction

Preceding sections have discussed direct effects of endophyte toxins on reproduction, that is, direct actions of toxins on hormone receptors and tissues associated with reproduction. In addition to these direct effects, endophyte toxins may reduce reproductive performance indirectly via physiological activities not directly associated with reproduction.

8.1. Heat stress

Heat stress is a common symptom in animals grazing E+ tall fescue and E+ perennial ryegrass pastures. Cattle grazing E+ tall fescue in North America frequently have elevated body temperature (Hoveland *et al.,* 1983; Hemken *et al.,* 1981; Osborn *et al.,* 1992) and, likewise, cattle grazing E+ perennial ryegrass in New Zealand often suffer high body temperatures, which result in heat stress (Easton *et al.,*1996).

Sheep grazing E+ pastures have also shown increased core body temperature. Fletcher (1993) showed that lambs grazing E+ perennial ryegrass had significantly higher rectal temperature than lambs grazing E- ryegrass under conditions of high ambient temperature.

Unlike cattle and sheep, horses grazing E+ pasture do not appear to suffer from heat. This is probably due to their ability to sweat and hence dissipate excess heat more effectively (Monroe *et al.,* 1988; Putnam *et al.,* 1991).

It is believed that the ergopeptine alkaloids play a major role in causing heat stress in animals grazing E+ pastures. These alkaloids reduce the ability of the animal to dissipate excess heat by their vasoconstriction activity (Rhodes *et al.,* 1991; Browning & Leite-Browning, 1997). Osborn *et al.* (1992) found a reduction in peripheral temperatures in cattle fed E+ tall fescue and ergotamine tartarate in thermoneutral conditions (21°C) and in a heat-stressed environment

(32°C), due to reduced blood flow to these areas. Gadberry *et al.* (1997) found that lambs fed diets containing different ergovaline concentrations showed a linear decline in skin temperature with increasing levels of ergovaline. At the same time, the actions of alkaloids on α-adrenergic receptors (Nolan *et al.*, 1986) and 5-HT_2 receptors (Genicot *et al.*, 1993) in the lungs cause vasoconstriction and bronchoconstriction which complicate the loss of heat by this organ (Oliver, 1997). Heat stress manifests itself in animals grazing E+ forage when environmental temperature is high. When environmental temperatures are low, there may be no effect of grazing E+ pasture on body temperature. Stamm *et al.*, (1994) observed no effect on body temperature in beef steers fed diets containing up to 475 ppb ergovaline in winter months.

It is likely that, under periods of high ambient temperature, animals grazing E+ pastures will suffer from heat stress and this could impact adversely on reproductive performance. The detrimental effects of elevated body temperature on reproductive performance have been well documented. Cockrem & McDonald (1969) found that increased body temperature in ewes decreased embryo implantation and the consequent number of lambs born. A study by Thwaites (1971) showed that the sheep embryo is very sensitive to maternal heat stress during the period immediately after mating. Cyclic ewes with hyperthermia exhibited less behavioural oestrus, had significantly lower serum progesterone levels and 6-fold higher prolactin levels than thermoneutral ewes (Hill & Alliston, 1981). Heat stress in ewes in the later stages of pregnancy has caused reduction in lamb birth weight (Brown *et al.*, 1977; Hopkins *et al.*, 1980).

Effects of elevated body temperature on ram fertility have also been reported. The association of rises in testicular temperature with reduced semen quality and testicular function have been well established (Moore & Oslund, 1924; McKenzie & Berliner, 1937; Fowler & Kennedy, 1967).

Heat stress has also resulted in reduced reproductive rates in cattle. Dunlap & Vincent (1971) reported that an increase in rectal temperature from 38.5 to 40°C postinsemination reduced pregnancy rates from 48% to 0%. As in the sheep, most embryonic mortality occurs soon after mating (Monty & Racowsky, 1987). Gwazdauskas *et al.* (1981) showed that heat-stressed cows had shorter oestrus, lower oestradiol levels, and the mean corticoid response to

corticotrophin was smaller, earlier to peak and of shorter duration. However, there was no difference in mean plasma progestins, LH, prolactin or corticoid levels which is in contrast to results obtained in ewes.

8.2. Nutrition

Cattle grazing E+ tall fescue have lower average daily gains (ADG) in liveweight than cattle grazing E- tall fescue (Williams *et al.*, 1984; Crawford *et al.*, 1989; Essig *et al.*, 1989; Paterson *et al.*, 1995). Sheep grazing E+ tall fescue have lower ADG than sheep grazing E- tall fescue (Chestnut *et al.*, 1992; Debessai *et al.*, 1993) and similarly, grazing E+ perennial ryegrass has resulted in lower ADG in sheep (Fletcher & Barrell, 1984; Fletcher *et al.*, 1999). Decreased weight gains have also been observed in rats and mice fed E+ diets (Varney *et al.*, 1987; Varney *et al.*, 1991b; Neal & Schmidt, 1985).

There are many possible mechanisms by which endophyte toxins may reduce animal weight gain, some of which are now considered.

8.2.1. Feed intake and grazing behaviour

There is evidence that the presence of endophyte and/or endophyte toxins in the diet reduces feed intake and affects grazing behaviour. It has been reported that cattle grazing E+ tall fescue have reduced feed intake (Stuedemann *et al.*, 1989). Dairy heifers fed E+ tall fescue hay had lower feed intakes than heifers fed E- tall fescue hay (Jackson *et al.*, 1988). Cosgrove *et al.* (1996) observed that weaner cattle grazing E- perennial ryegrass pasture consumed more dry matter (DM) than those grazing E+ perennial ryegrass pasture.

Lower feed intake has also been observed in geldings and yearling horses fed E+ tall fescue hay compared with those grazing E- tall fescue hay (Redmond *et al.*, 1991; McCann *et al.*, 1992).

Fletcher & Barrell, (1984) suggested that reduced growth in sheep grazing E+ perennial ryegrass may have been due to reduced herbage intakes. Chestnut *et al.* (1992) showed that hay (DM) intake was greater for lambs fed E- tall fescue hay than for lambs given E+ tall

fescue hay and Aldrich *et al.* (1993a) found that *ad libitum* DM intake was lower in lambs fed E+ tall fescue than lambs fed E- tall fescue.

In addition to reductions in feed intake, animals grazing E+ pastures have been observed to differ in grazing behaviour. Howard *et al.* (1992) found that steers grazing E+ tall fescue displayed altered day-time grazing behaviour as well as a reduction in voluntary intake. Coffey *et al.* (1992) found that cattle grazing E+ tall fescue pastures grazed for the same length of time as cattle on E- tall fescue pasture, but spent more time grazing during night hours. Cattle grazing E+ tall fescue pasture show a higher degree of selectivity (Barth *et al.*, 1991). Differences in grazing preference for E+ and E- pasture have been demonstrated in sheep. Edwards *et al.* (1993) showed that, although grazing time was similar for hoggets grazing E+ and E- perennial ryegrass, less E+ was consumed possibly owing to a reluctance to graze the E+ pseudostem horizon. In palatability tests it was shown that E- tall fescue hay was preferred by sheep compared to E+ tall fescue hay (Samford-Grigsby *et al.*, 1997).

8.2.2. Forage digestibility

There is some evidence to suggest that the presence of endophyte affects the relative digestibility of feed. Aldrich *et al.* (1993a) found that digestibility of E+ tall fescue was lower than that of E- tall fescue in lambs and similarly, Aldrich *et al.* (1993b) found that the DM and organic matter (OM) digestibilities were 9% lower for E+ than E- tall fescue in cattle. Hannah *et al.* (1990) found that in sheep fed a diet with or without ergovaline, rumen and total tract OM, neutral detergent fibre (NDF) and cellulose digestibilities were less when diets contained ergovaline. However, Stamm *et al.* (1994) found that apparent DM digestibility and total tract NDF digestion were not influenced by ergovaline concentration although it should be noted that no other symptoms of endophyte toxicosis were observed in this trial.

8.2.3. Effects of endophyte on the gastrointestinal (GI) system.

Some endophyte toxins have been found to affect GI smooth muscle. Smith *et al.* (1997) found that the fungal tremorgens penitrem, paxilline and lolitrem B caused variable amounts of stimulation and inhibition in the smooth muscle of the antrum and duodenum. The effects of the ergopeptine alkaloids on GI smooth muscle are yet to be examined, however, it is

possible that these toxins will interfere with reticulo-rumen contractions, smooth muscle tone in the GI tract via adrenergic, serotonergic and dopaminergic activity (Oliver, 1997).

9. Production of the major endophyte toxins in E+ perennial ryegrass pasture

There are only a few reports to date on the production of toxins from endophyte-infected perennial ryegrass either in controlled conditions or in grazed pastures. Currently studies are being conducted into the many factors that may control toxin production. There are strong indications that seasonal and environmental conditions play an important role and it has also been discovered that there are distinct patterns of toxin distribution within the ryegrass plant.

9.1. Lolitrem B

The alkaloid lolitrem B is present in perennial ryegrass plants only when they are infected with *Neotyphodium lolii*. Concentrations of lolitrem B are much higher in the leaf sheath than in the leaf blade and increase progressively from blade tip to lower sheath (Keogh *et al.*, 1996). Peak lolitrem B production in E+ perennial ryegrass pastures is in late summer and autumn (Ball *et al.*, 1997), which is why this is the most common time for ryegrass staggers problems.

9.2. Ergovaline

Ergovaline levels increase from spring when seed head emergence starts and the compound continues to accumulate in the pasture to peak in late summer and autumn, similarly to lolitrem B (Ball *et al.*, 1995). Ergovaline is also distributed in the plant similarly to lolitrem B with higher concentrations in the leaf sheath and seed head than in the leaf blade (Keogh *et al.*, 1999)

Lane *et al.* (1997a) found that added nitrogen or water stress is associated with elevated ergovaline production in perennial ryegrass pastures. Levels of ergovaline above 0.5 ppm may be commonplace in New Zealand ryegrass pastures (Easton *et al.*, 1996) and extreme levels up to 27 ppm have been measured in natural associations of endophyte-infected perennial ryegrass under glasshouse conditions (Lane *et al.*, 1997b).

10. **Purpose and scope of the study**

This study aimed to examine the effects of grazing perennial ryegrass pasture infected with the endophyte fungus *Neotyphodium lolii*, on reproductive performance in ewes and elucidate the major mechanisms involved. The major aspects of reproductive performance examined were the oestrous cycle, mating, gestation and parturition. Additionally, as integral parts of reproductive performance, mammogenesis, lactation, and performance of offspring were also examined. Finally, feed intake was examined as the possible cause of lower liveweight in animals grazing E+ pasture, and its relationship with reproductive performance of the ewe.

This involved a series of grazing trials that measured endophyte toxin levels in ryegrass pastures during the year and determined their relationship with the aspects of reproductive performance of the grazing ewe mentioned above. More intensive trials were conducted to examine the effects of the major endophyte toxin ergovaline on endocrine systems regulating important reproductive functions such as the oestrous cycle and lactation.

The specific objectives of the experiments involved in this programme were:

1) To compare the reproductive performance of ewe flocks maintained on either endophyte-infected or endophyte-free perennial ryegrass pasture.
2) To measure the growth rate of lambs, born to ewes that were maintained on either endophyte-infected or endophyte-free ryegrass pasture.
3) To examine the effects of long-term grazing of endophyte-infected ryegrass on progesterone, prolactin and luteinizing hormone levels in cyclic ewes.
4) To determine the effects of the endophyte toxin ergovaline and environmental temperature on serum prolactin, progesterone, cortisol and insulin levels in ewes during late pregnancy and parturition.
5) To determine the effects of endophyte presence in pasture on feed intake of ewes and suckling lambs.

When examining and discussing the various toxic effects of grazing E+ ryegrass pasture, this study focuses on the endophyte toxin, ergovaline. The reasons for this were that ergovaline is the most abundant toxin produced by the ryegrass endophyte (with the exception of lolitrem B), and that much of the research conducted has also focused on ergovaline as being the major toxin responsible for endophyte toxicosis. However, it needs to be stressed that other ergopeptine alkaloids and several lysergic acid amides, which are physiologically active in mammals, are also produced in significant amounts by N. lolii and N. coenophialum. Therefore, it is likely that many, if not all of these other toxins may either contribute to or cause some of the toxic effects seen in animals grazing E+ pastures.

CHAPTER III

Reproductive performance of ewes grazing either endophyte-infected or endophyte-free perennial ryegrass

1. Introduction

Reduced reproductive performance of animals grazing tall fescue infected with the endophyte *Neotyphodium coenophialum* has been well documented (Porter & Thompson, 1992). Most reports were on horses or cattle, and only a small number have examined the effects of endophyte-infected tall fescue on the reproductive performance of sheep. Grazing endophyte-infected tall fescue has reduced reproductive rates (Bond *et al.,* 1988), ovulation rate and lambs carried to 90 days of pregnancy (Kramer *et al,.*1999) in sheep.

At present there is a lack of research into the effects on reproductive performance of grazing perennial ryegrass infected with *Neotyphodium lolii.* Endophyte-infected perennial ryegrass pastures have been found to produce similar toxic alkaloids to endophyte-infected tall fescue (Rowan *et al.,* 1990). Therefore, it is likely that the reproductive performance of sheep grazing endophyte-infected perennial ryegrass may be affected in a similar manner to that of sheep grazing endophyte-infected tall fescue. The only reported effect of grazing endophyte-infected perennial ryegrass to date is a delay in the onset of lambing in ewes (Eerens *et al.,* 1997).

Perennial ryegrass is a widely used pasture species in New Zealand. Ryegrass pastures throughout New Zealand have regularly been found to be infected with *N. lolii* (Prestidge *et al.,* 1985; Wedderburn *et al.,* 1989; Widdup & Ryan, 1992). The importance of perennial ryegrass to New Zealand's pastoral industry underscores the need to examine the effects of grazing these infected pastures on animal reproductive performance and to determine what, if any, production losses are incurred.

The objectives of this trial were to determine the effects of long term grazing of endophyte-infected perennial ryegrass on reproductive performance of the ewe, and identify some of the possible mechanisms involved.

2. Materials and methods

2.1. Animals and treatments

2.1.1. Animals

Rising two-tooth, virgin, Romney ewes (rising 2 year olds), of a similar genetic background, were obtained from a Northland flock and transported to AgResearch Aorangi Lowland Research (AOR) Station in the Manawatu on 11 January 1996. The ewes grazed a brassica crop until 11 March 1996 when all ewes were ear-tagged, and seventy-eight ewes randomly selected and allocated to two treatment groups with the same average liveweight. Ten spare ewes were allocated to each group as replacements for animals in the treatments groups if required.

Two-tooth ewes were used to enable the reproductive performance to be determined for the same ewes over a number of years. This study focuses on the first two years of an ongoing four-year trial.

2.1.2. Treatment pastures

Six 0.5 ha plots were fenced and sown on 8 November 1995 at AOR on Kairanga silt loam. The trial area had a history of lotus (*Lotus pedunculatus*) and cocksfoot (*Dactylis glomerata*) pastures.

Three replicate plots were sown in Nui perennial ryegrass seed (33 kg/ha) infected with high levels (reputed 88%) of wild-type endophyte (E+). The remaining three replicate plots were sown in Nui perennial ryegrass seed (40 kg/ha) which was free (reputed 0%) of endophyte infection (E-). The pasture replicates were arranged in a complete randomised design across the trial area. On 4th December 1995 the newly sown trial area was sprayed with 2.5 l/ha MCPB, 2.5 l/ha 2-45D, and 5 l/ha MCPA to remove any non-grass species.

In October 1996 a further two 0.5 ha plots were sown in Nui E+ and two in Nui E- seed (25 kg/ha). These additional plots were used to graze the spare ewes and for additional feed when required by the trial groups.

2.1.3. Fertiliser applications

In addition to maintenance applications of Nitrogen (N), Phosphorus (P), Potassium (K) and Sulphur (S), applications of N were used on several occasions as a management tool to maintain pasture growth (Table 3.1.).

Table 3.1. Fertiliser applications made during the pre-trial period and during 1997 and 1998.

MONTH/YEAR	FERTILISER TYPE	RATE (kg/ha)	N,P,K,S (kg/ ha)
April' 96	Urea	140	64,0,0,0
	Super Phosphate	200	0,18,0,22
August' 96	Urea	80	37,0,0,0
November' 96	Urea	120	55,0,0,0
January' 97	Urea	100	46,0,0,0
February' 97	Ammonium Sulp.	50	11,0,0,12
September' 97	Urea	160	74,0,0,0
March' 98	Urea	160	74,0,0,0
August' 98	Urea	80	37,0,0,0
September' 98	Urea	160	74,0,0,0
November' 98	Urea	100	46,0,0,0

2.1.4. Flock management.

Each group of ewes was allocated to one of the two pasture treatments and was used to graze their respective pastures from the end of March 1996 to January 1997 as a pre-trial preparation. The spare ewes were randomly divided into two even groups and grazed on adjacent E+ or E- pastures.

All trial and spare ewes were mated in April 1996 and lambed on their respective areas in September. The lambs were weaned in November 1996 and removed from the trial.

By the end of 1996 the ewes grazing the E- pasture were significantly heavier than the E+ ewes, so on 10 January 1997 spare ewes were recruited to replace some ewes in the treatment groups so that the groups were the same average liveweight at mating in 1997. The trial period began at mating in 1997 and from this time the same ewes were used in each group throughout the 2-year trial period, and were replaced with spare ewes only in the case of death. The entire trial period was intended to run from January 1997 to Mating in 2000 so that the long-term performance of the ewes could be measured. However, this study is focused on data collected during the period between January 1997 and March1999.

In 1997 and 1998 the groups of ewes were rotationally grazed with the same pasture allowance, from the end of lamb docking in late September until the end of mating in April. The extra plots sown in 1996 were used to provide additional feed to groups that required it during rotational grazing and any surplus pasture, caused by differences in feed intake between the treatment groups, was accumulated on these extra plots and grazed by the spare ewes. The spare ewes were also used to graze the main plots to maintain uniformity between the E+ and E- pastures. Mature seed head and long ungrazed areas of the pastures were removed by a forage harvester in late summer to maintain pasture quality, with thorough cleaning of the machinery between plots to ensure no seed contamination.

On the 1 March each year a Suffolk ram fitted with a mating harness was introduced to each group of ewes. The rams were rotated between the groups weekly for two oestrous cycles and then removed.

From the end of mating, each group of ewes was set stocked over treatment plots throughout gestation and lambing.

Each year the ewes were vaccinated with Multiline™ 5 in 1 against clostridial diseases, and crutched two weeks prior to the start of lambing. In 1997, lambs remained with the ewes until weaning at the beginning of November (11 weeks from start of lambing) at which point they were removed from the trial. In 1998, lambs were kept with the ewes 6 weeks longer and weaned in mid December (17 weeks from start of lambing) so that growth rates could be monitored further.

In late September all lambs had elastrator™ rubbers rings put on their tails and were vaccinated against clostridial diseases. At this time all ewes and lambs were drenched with an anthelmintic and lambs were subsequently drenched every six weeks until weaning.

The ewes were shorn in November each year and ewes that died during the year were replaced with the spare ewes. All ewes are treated with Seraphos™ dip in February to prevent flystrike.

2.2. Sampling

2.2.1. Animal measurements

Rams were equipped with a tupping harness and crayon to enable the determination of mating date for each ewe. After the 1st oestrous cycle, the crayon colour was changed and ewes which returned to oestrus were recorded.

The ovaries of each ewe were examined by laparoscopy between days 3 and 7 after first mating in 1997 and 1998 to measure ovulation rate (corpora lutea/ ewe). All ewes were also examined by laparoscopy after first mating in the pre-trial period in 1996.

All ewes were examined by ultra-sonography 90 days from the start of mating to measure the number of foetuses carried per ewe. All ewes were also pregnancy scanned in 1996 and in 1999.

Two weeks prior to lambing, neck tags were attached to all ewes to enable identification from a distance during lambing. After each ewe lambed, the lambs were tagged and the number of lambs born per ewe, birth date, birth weight, sex and any deaths were recorded. In 1997, lambs were weighed weekly for the first six weeks of life and at weaning (11 weeks). In 1998, lambs were weighed every 2-3 weeks from birth to weaning (17 weeks).

On 1 September 1998 single-rearing ewes (n=5, and 7 for the E+ and E- groups respectively) and 6 twin-rearing ewes of similar lambing date were selected from each group. These ewes were separated from their lambs and milked by a method described by Peterson (1992) at 0900 am and 1500 pm on one day every week for 12 weeks to determine daily milk

production. This was repeated using 7 twin-rearing ewes from each group on weeks 1, 3 and 5 of lactation in 1998.

Each group of ewes was weighed monthly throughout the year and fleece weights were recorded annually at shearing.

During the summer and autumn all ewes were yarded and left in the shade for half an hour after which rectal temperatures were measured. A blood sample was collected by jugular venipuncture from all ewes before mating (end of February) and weekly during lactation to measure serum prolactin levels.

The E+ group was assessed regularly during the summer and autumn for RGS using a severity scale devised by Keogh (1973).
Ewes were scored for faecal soiling (dags) (See Plate 3.1.) by eye in summer and autumn each year using a scale of 0 (clean) to 3 (heavy faecal soiling). Dags were collected from animals representing each score. The dags were weighed to give an average weight (± SEM) for each score (0 = 0 g, 1 = 99 ± 11g, 2 = 208 ± 14 g and 3 = 389 ± 71 g).

Ewes that died on the trial, where cause of death was not immediately apparent, were examined post-mortem by J.S. Lumsden, Department of Veterinary Pathology and Public Health, Massey University (see appendix I for pathology reports).

In March 1996 urine samples were collected from 10 randomly selected ewes in each group to determine urinary zearalenone levels. (zearalenone is an oestrogenic fungal metabolite produced by *Fusaria* species commonly found in ryegrass pastures and has been found to reduce reproductive performance in sheep (Kramer, 1997)). The urinary analysis was used as an indication of any differences in zearalenone levels between the E+ and E- pastures. Urinary zearalenone was analysed by ELISA immunoassay (di Menna *et al.*, 1991).

2.2.2. Pasture measurements

Three quadrat pasture cuts (20 cm x 50 cm) were taken monthly from under exclusion cages, and grazed areas in each plot. After each sampling the exclusion cages were moved to a new

grazed area of the pasture. Each pasture sample was freeze-dried and weighed. The difference in DM between the pasture cut from under the exclusion cage and the grazed pasture cut taken at the previous sampling from the same plot was used to estimate kg DM/ha grown daily during the month.

A subsample of each pasture cut taken was dissected into ryegrass leaf-blade and leaf-sheath, and non-ryegrass species. The ryegrass components were ground and analysed for lolitrem B and ergovaline concentration. A further pasture subsample was taken and pooled across replicates within a treatment. The pooled samples were ground and used for nutritional analysis.

Plate 3.1. Faecal soiling of the wool around the crutch, commonly referred to as dags.

2.3. Endophyte infection levels

Fifty ryegrass tillers were taken from each treatment plot during summer each year. Epidermal sections were removed from each tiller, stained with lactophenol blue, and examined with a light microscope for the presence of endophyte hyphae.

2.4. Serum prolactin analysis

Serum prolactin levels were determined using a double-antibody ^{125}I radioimmunoassy procedure based on the methods of Van Landegham & Van de Weil (1978) at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand (see Chapter VI, section 2.1.2. for details).

2.5. Herbage ergovaline and lolitrem B analyses

Freeze-dried and ground ryegrass samples were measured for ergovaline and lolitrem B levels by HPLC as described by Barker *et al.* (1993) at the Plant Biochemistry Laboratory, AgResearch Grasslands, Palmerston North.

Ergovaline

Herbage samples (50 mg) with added ergotamine tartrate internal standard (less than 1 µg in 50 µl of methanol) were extracted with 1 ml of chloroform-methanol-ammonia (concentrated solution), (75:25:2 v/v) overnight at room temperature in darkness.

The extraction solvent was separated by aspiration through an immersion filter and the plant residues rinsed with two further 0.5 ml portions of solvent. The combined extract was evaporated to dryness under vacuum without heating and redissolved and suspended in 0.5 ml carbon tetrachloride and 0.5 ml tartaric acid solution (25 mM in 50% v/v aqueous methanol). The phases were allowed to separate on standing, and the aqueous phase transferred with filtration into a vial for HPLC sampling.

Ergovaline was separated out by reverse phase HPLC (Brownlee RP-18 column, 5 µm, 100 x 4.6 mm with RP-18 Newguard precolumn) at 30°C with a gradient solvent system of 0.1 M

ammonium acetate in water and acetonitrile (25 % v/v acetonitrile to 50 – 60% acetonitrile with a concave 35 – 45 min gradient; flow 1 ml/min) and measured by fluorescence detection (λ_{ex} 310 nm, λ_{em} 410 nm)

Lolitrem B

Herbage samples (50 mg) were extracted for 1 h with 1 ml of chloroform-methanol (2:1 v/v). The extraction solvent was separated by aspiration through an immersion filter and the plant residues rinsed with two further 0.5 ml portions of solvent. The solvent was evaporated without heating under reduced pressure and the residues taken up in 1 ml 1,1-dichloroethane:acetonitrile (4:1 v/v). The extract was transferred through a syringe filter into vials for HPLC sampling.

Lolitrem B was measured following HPLC separation using a silica column (Brownlee, 220 x 4.6 mm) with a solvent phase of dichloromethane, acetonitrile, and water (840:160:1 v/v) at 1 ml/min. Fluorescence detection was with excitation at 268 nm and emission at 440 nm and the amount of lolitrem B estimated by comparison of the integrated peak area with those of reference standards.

2.6. Pasture nutritional analysis

Freeze-dried and ground pasture samples were assayed by NIR for protein, lipid, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble carbohydrate (CHO), organic matter digestibility (OMD), and metabolisable energy (ME) at the feed-TECH laboratory, AgResearch Grasslands, Palmerston North.

2.7. Statistical analyses

All statistical analyses were carried out using Graph pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.

Ovulation rate, lambs carried per ewe at scanning, mating date, lambing percentage and faecal soiling data were analysed by chi-squared test. Ewe liveweight data and lamb growth rate data were analysed by fitting a generalised linear model (GLM) to determine sources of

variation due to treatment (endophyte), time (week/month of weighing) and treatment X time interactions. Linear regression analysis was conducted to determine relationships between ovulation rate and ewe liveweight, daily milksolid production and lamb growth rate, cumulative milksolid yield and lamb liveweight. Fleece weight data were analysed by t-test. Serum prolactin levels at mating and urinary zearalenone levels were analysed by t-test. Serum prolactin levels and daily milksolid production during lactation were analysed by GLM to determine variation due to endophyte, weeks after parturition and endophyte X week interaction. Rectal temperature data were analysed by analysis of variance (ANOVA).

Mean (±SEM) pasture growth rates, ergovaline and lolitrem B levels were calculated across the three treatment pasture replicates and were analysed by GLM to determine variation due to endophyte status, month of sampling and endophyte X month interaction. Pasture nutrition parameter means were calculated by pooling results within seasons and across years. Each nutritional parameter was analysed by GLM to determine variation due to endophyte, season and endophyte X season interaction.

3. Results

3.1. Animal measurements

3.1.1 . Reproductive performance and ewe mortality

Reproductive parameters measured in the pre-trial period (1996) and during the first two years of the trial are presented in Table 3.2.

There was a significant difference in mean number of days from introduction of the ram to mating with the E+ ewes being mated 1.8 days later than E- ewes (Table 3.3.). There were no significant differences in any other of the reproductive parameters measured between the E+ or E- ewes in 1997 or 1998. However, there was a significantly ($P<0.05$) higher proportion of dry ewes at scanning in the E+ than in the E- group (33% (12/36) vs 14% (5/36)) in 1999. Lambing started around 24 July and was completed by 10 September in each year.

Ewe mortality was generally low (5%/year) and there were no significant differences in the mortality rate of ewes between each treatment. Ewe deaths during the trial period (March 1997 to December 1998) were 5 and 4 for the E+ and E- groups respectively. (Details of ewe deaths are summarised in appendix I, Table 1.1.)

Table 3.2. Ovulation rate, % lambs carried at scanning, % of ewes that returned to oestrus and that were dry at scanning, and % lambs born and % weaned per ewe mated in the E+ and E- groups

	Pre-trial (1996)		**1997**		**1998**	
	E+	**E-**	**E+**	**E-**	**E+**	**E-**
Ovulation rate (n = 39/group)	1.53 + 0.08	1.43 + 0.08	1.67 ± 0.08	1.63 ± 0.08	1.66 ± 0.08	1.81 ± 0.08
Number of ewes that returned to oestrus	11	8	8	11	8	9
Scanning % (includes dry ewes)	125 + 8	131 + 10	146 ± 10	163 ± 7	141 ± 10	136 ± 9
Number of ewes dry at scanning	1	2	2	1	2	4
Lambing % (born/ewes mated)	120 + 8*	126 + 9*	145 ± 10	161 ± 9	141 ± 10	136 ± 9
Lambing % weaned (per ewes mated)	102 + 10	103 + 10	104 ± 11	122 ± 10	117 ± 10	122 ± 9

*Lower Lambing % compared with scanning % was most likely due to errors distinguishing between single and twin-bearing ewes at scanning.

Table 3.3. Mean (±SEM) number of days from the start of mating until ewes in the E+ and E- groups were mated in each year (1997, 1998).

YEAR	**E+**	**E-**
1997	7.7 ± 0.6[a]	5.9 ± 0.5[b]
1998	7.0 ± 0.7[a]	5.3 ± 0.5[b]

Means with different superscript letters within a row differ significantly (P<0.05)

3.1.2. Long-term reproductive performance of the ewe

The mean total number of ovulations (during the first cycle of mating) (1996 to 1998, NB: ovulation rates were not measured in 1999), number of lambs carried at pregnancy scanning (1996 to 1999) and number of returns to service (1996 to 1999) for ewes in the E+ and E- groups are presented in Table 3.4.

The parameters were calculated by adding data for the pre-trial year and each subsequent year of the trial from ewes that had been present in each treatment group throughout the pre-trial and trial period (n=25 for both groups) (March 1996 to June 1999). There were no significant differences in the total number of ovulations, lambs carried or returns to oestrøus for the 4-year period between the E+ and E- groups.

The proportions of the total number of pregnancies in each group between 1996 and 1999 that were single-bearing or multiple-bearing, and of dry ewes at scanning are presented in Table 3.5.

There were significantly ($P<0.06$) more dry ewes at scanning in the E+ group than in the E- for the 4-year trial period. There were no significant differences in the numbers of single- and multiple-bearing ewes between the E+ and E- groups.

Table 3.4. Mean (±SEM) total number of ovulations, lambs carried at scanning and returns to oestrus per ewe for the E+ and E- groups between 1996 and scanning in 1999.

	E+	**E-**
Total ovulations/ewe	5.36 ± 0.32	5.72 ± 0.31
Total lambs carried/ewe	4.88 ± 0.19	4.92 ± 0.20
Total returns to oest./ewe	1.12 ± 0.16	1.21 ± 0.18

Table 3.5. Proportions of the total numbers of pregnant ewes that were single-bearing, multiple-bearing and the dry ewes in the E+ and E- groups between 1996 and scanning in 1999.

	E+	E-
DRY%	14	6*
SINGLE-BEARING%	40	45
MULTIPLE-BEARING%	46	49

* proportions in the same row differ significantly (P<0.06)

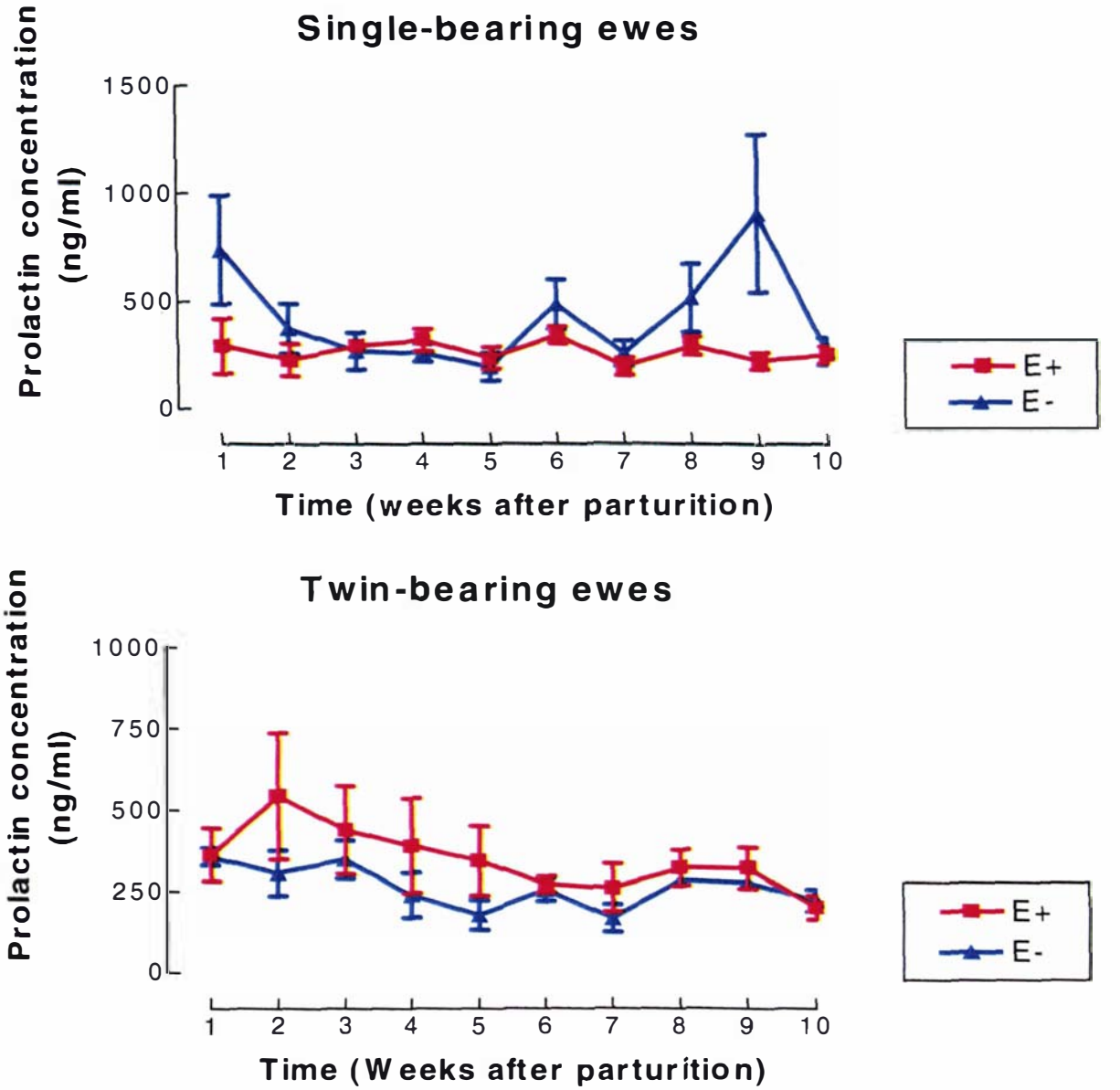
3.1.3. Serum prolactin

Serum prolactin levels were significantly (P<0.02) lower in the E+ (34.0 ± 5.8 ng/ml) than in the E- group (82.6 ± 15.4 ng/ml) prior to mating in 1997. The difference in serum prolactin levels between E+ (90.2 ± 16.7 ng/ml) and E- (136.5 ± 28.4 ng/ml) ewes prior to mating in 1998 was not significant (P=0.17).

During the first 10 weeks of lactation in 1998 there were no significant differences in serum prolactin levels between the E+ and E- single-rearing ewes at any sampling. However, mean serum prolactin levels over the entire 10-week period were significantly (P<0.05) higher in the E- single-rearing ewes than in the E+ single-rearing ewes (Figure 3.1.). There was no significant effect of time (week of lactation) nor interaction between time and endophyte on serum prolactin in either the E+ or E- single-rearing ewes.

Similarly there were no significant differences in serum prolactin levels between the E+ and E- twin-rearing ewes at any one sampling. However, mean serum prolactin levels in twin-rearing ewes were significantly (P<0.05) higher in the E+ ewes than the E- ewes over the entire 10-week period (Figure 3.1.). Data analysis on twin-rearing ewes suggested prolactin levels fell as lactation progressed (P=0.15) and that an interaction existed between endophyte and week of lactation (P=0.19).

Figure 3.1. Mean (±SEM) serum prolactin levels of single and twin-rearing ewes grazed on either E+ or E- ryegrass during lactation in 1998.



3.1.4. Ewe liveweight

The liveweight data for ewes, classified relative to the number of lambs reared each year in 1997 and 1998 is presented in Table 3.6. Due to the small number of dry ewes each year, their liveweight was not significantly different from any of the other ewes of different rearing status in either group.

The liveweight of twin- and single-rearing ewes grazing the E+ pasture was significantly ($P<0.0001$) lower than the ewes of similar rearing status grazing the E- pasture during the 1997 and 1998 trial period.

Month of the year was a significant ($P<0.0001$) source of variation in ewe liveweight during 1997 and 1998 in both the twin- and single-rearing ewes of each group.

The month X treatment interaction effect on the liveweight was significant ($P<0.0001$) for single-rearing ewes and was close to significant ($P=0.08$) for the twin-rearing ewes.

At the start of mating (1 March) in 1997 there was no significant difference in liveweight between ewes grazing E+ or E- pasture (54.7 $\pm$ 0.8 kg and 55.5 $\pm$ 0.7 kg for E+ and E- respectively). The liveweight data are divided into subgroups of ewes that subsequently reared either twin (n=22 and 26 for E+ and E- groups respectively) or single (n=15 and 12 for E+ and E- groups respectively) lambs in 1997. Twin-rearing ewes in the E+ group were significantly ($P<0.05$) lighter than twin-rearing ewes in the E- group.

At the end of mating (10 April) in 1997 there were no significant differences in the liveweight of any subgroup of ewes. There were no significant liveweight changes in any group of ewes during mating.

During gestation (end of mating to 24 July) in 1997 the twin- and single-rearing ewes in both the E+ and E- groups put on significant amounts of weight, some of which would have been associated with conceptus development.

By the start of lambing (24 July) the twin-rearing ewes in the E- group were significantly ($P<0.05$) heavier than all other groups of ewes. The groups of ewes were not weighed in October or November 1997, however, the December 1997 weighing indicated that liveweight differences between the groups were maintained throughout lactation. It also appeared that twin-rearing ewes lost significantly more weight during lactation than single-rearing ewes in both treatment groups.

Liveweight data for 1998 are divided into ewes that reared either twin (n=19 and 17 for E+ and E- groups respectively) or single (n=18 for both E+ and E- groups) lambs in 1998. Mean ewe liveweight for the ewes of different rearing status did not alter significantly when the data sets were redefined at the start of 1998.

Between January 1998 and February 1998 the liveweight of single-rearing ewes was significantly ($P<0.05$) lower in the E+ group than in the E- group and there were no significant differences in the liveweight of twin-rearing ewes between the E+ and E- groups.

At the start of mating (1 March) in 1998 the twin and single-rearing ewes in the E+ group were significantly ($P<0.05$) lighter than their E- counterparts. During the mating period (1 March to 12 April, 42 days), ewes that subsequently reared twin lambs in the E+ group gained significant ($P<0.05$) weight (4.4 ± 0.2 kg) whereas the liveweight of the E- twin-rearing ewes did not change significantly. During the mating period the E+ ewes that subsequently reared single lambs gained weight (2.8 ± 0.2 kg, (not significant)), however between the end of mating and the May 1998 weighing these ewes lost significant ($P<0.05$) weight (3.5 ± 0.2 kg). The E- single-rearing ewes lost weight on average during mating (2.9 ± 0.2 kg (not significant)) and the liveweight of these ewes did not change significantly between the end of mating and the May weighing.

The E- and E+ single-rearing ewes and the E- twin-rearing ewes maintained their liveweight from the end of mating to the July weighing, which incorporated most of gestation, whereas the E+ twin-rearing ewes lost weight towards the end of gestation (June 1998 to July 1998). This trend continued through lactation (July 1998 to December 1998). Single-rearing ewes in the E+ group were an average 10.1 ± 0.7 kg lighter than the single-rearing ewes in the E- group between August 1998 and February 1999. There were no significant differences in the liveweight of twin-rearing ewes between the E+ and E- groups during this period.

Table 3.6. Mean (±SEM) liveweight (kg) of twin and single-rearing ewes grazing either E+ or E- perennial ryegrass pasture in 1997 and 1998.

	TWIN-REARING EWES		SINGLE-REARING EWES	
MONTH 1997	**E+**	**E-**	**E+**	**E-**
MAR	54.3 ± 1.2^{a}	59.4 ± 1.1^{b}	55.5 ± 1.0^{ab}	53.4 ± 0.9^{a}
APR	56.3 ± 1.5	56.4 ± 1.1	57.2 ± 1.4	55.3 ± 0.7
MAY	57.8 ± 1.2^{ab}	57.4 ± 0.8^{ab}	59.0 ± 0.9^{a}	54.4 ± 0.7^{b}
JUN	N/S	N/S	N/S	N/S
JUL	60.0 ± 1.2^{a}	65.7 ± 0.9^{b}	60.1 ± 1.0^{a}	62.5 ± 1.1^{ab}
AUG	65.3 ± 1.0^{a}	69.2 ± 1.0^{b}	64.9 ± 1.0^{a}	67.4 ± 1.0^{ab}
SEP	61.2 ± 1.1^{a}	70.2 ± 1.1^{b}	61.4 ± 1.4^{a}	67.4 ± 1.5^{b}
OCT	N/S	N/S	N/S	N/S
NOV	N/S	N/S	N/S	N/S
DEC	58.4 ± 1.2^{a}	65.8 ± 1.9^{b}	62.4 ± 1.8^{ab}	67.2 ± 1.8^{b}
		1998		
JAN	58.3 ± 1.2^{a}	63.5 ± 2.6^{ab}	61.8 ± 1.6^{a}	66.8 ± 1.8^{b}
FEB	58.1 ± 1.2^{a}	62.9 ± 2.6^{ab}	61.6 ± 1.6^{a}	67.1 ± 1.8^{b}
MAR	56.9 ± 1.9^{a}	63.1 ± 1.4^{bc}	62.1 ± 1.4^{ab}	67.8 ± 1.9^{c}
APR	60.1 ± 1.6	61.4 ± 1.5	64.9 ± 1.5	64.9 ± 1.6
MAY	65.3 ± 1.7^{ab}	67.4 ± 1.6^{a}	60.4 ± 1.7^{b}	64.8 ± 1.4^{ab}
JUN	65.2 ± 1.6^{ab}	67.3 ± 1.4^{a}	60.8 ± 1.6^{b}	64.5 ± 1.4^{ab}
JUL	62.8 ± 1.6^{ab}	67.0 ± 1.4^{a}	59.6 ± 1.6^{b}	64.8 ± 1.6^{ab}
AUG	61.9 ± 1.6^{ab}	63.8 ± 1.4^{ab}	58.1 ± 1.6^{a}	65.6 ± 1.6^{b}
SEP	61.2 ± 1.6^{ab}	61.8 ± 1.3^{ab}	56.8 ± 1.6^{a}	65.9 ± 1.6^{b}
OCT	58.6 ± 2.7^{ab}	60.4 ± 2.6^{ab}	56.2 ± 2.0^{a}	67.9 ± 1.8^{b}
NOV	60.3 ± 2.7^{ab}	60.4 ± 2.5^{ab}	56.3 ± 2.0^{a}	65.2 ± 1.6^{b}
DEC	58.6 ± 2.0^{a}	62.1 ± 1.7^{ab}	54.1 ± 1.9^{b}	65.5 ± 1.3^{c}
		1999		
JAN	61.9 ± 1.7^{ab}	64.9 ± 1.8^{a}	55.4 ± 2.3^{b}	67.6 ± 1.4^{a}
FEB	61.4 ± 1.6^{a}	59.3 ± 1.8^{ab}	53.5 ± 2.1^{b}	63.3 ± 1.6^{a}

Means with different superscript letters within a row differ significantly ($P<0.05$)
N/S = weights were not taken in these months

3.1.5. Relationships between ewe liveweight at mating, liveweight change during mating and reproductive performance

Liveweight data for ewes relative to their reproductive performance in presented in Table 3.7. At mating in 1997 there were no significant differences in pre-mating ewe liveweight (LW) across treatments and ovulation rate.

In 1998, ewes that had either single or multiple ovulations in the E+ group were significantly ($P<0.001$) lighter than ewes with the same ovulation rate in the E- group. There were no significant differences in ewe LW between ewes that had either single or multiple ovulations in the E- group. However, ewes that had multiple ovulation in the E+ group were significantly ($P<0.01$) heavier than ewes that had single ovulation in the E+ group. Ewes in the E- group were significantly heavier ($P<0.01$) across ovulation rates in 1998 than they had been in 1997. Ewes that had multiple ovulations in the E+ group in 1998 were significantly heavier than ewes that had multiple ovulation in the E+ group in 1997. There were no significant LW differences between E+ ewes that had single ovulations in 1997 and those in 1998.

There was no significant difference in liveweight change from two weeks before to the end of mating (LWΔ) across treatments, years or ovulation rate.

In 1997 there were no significant differences in pre-mating ewe liveweight across treatment and scanning rank.

In 1998, LW of the E+ ewes was significantly ($P<0.001$) lower than the LW of the E- ewes of the same scanning rank. There were no significant differences in LW across scanning ranks in the E- group. Ewes that carried two lambs at scanning in the E+ group in 1998 were significantly heavier at mating than single-bearing and dry ewes in the E+ group. LW at mating of the E- ewes was significantly greater across all scanning ranks in 1998 than they had been in 1997. Ewes that carried two lambs at scanning in the E+ group were significantly heavier at mating in 1998 than they had been in 1997. There were no significant differences in the LW of single-bearing ewes in the E+ group between 1997 and 1998.
In 1997 there were no significant differences in LWΔ across treatments and scanning rank.

In 1998 E+ ewes gained weight during mating whereas E- ewes lost weight. There was no significant difference in LWΔ across scanning rank within a treatment group.

In 1997 there were no significant differences in LW across treatments and cycle of conception.

In 1998 ewes that conceived in the second cycle in the E- group were significantly heavier at mating than all ewes in the E+ group. There were no significant differences in any other groups across treatments and cycle of conception.

There were no significant differences in LWΔ across cycle of conception within treatment group in either 1997 or 1998.

In 1997 LW and LWΔ were not significant sources of variation in ovulation rate, number of lambs carried or scanning in either the E+ or E- group.

In 1998 LW and LWΔ were not significant sources of variation in ovulation rate in the E- group. However, LWΔ was a significant (13.6%, $P<0.05$) source of variation in the number of lambs carried at scanning. The regression equation of LW and LWΔ on the number of lambs carried at scanning (SC) in the E- group was:

$$SC = 1.14 + 0.0049\,LW + 3.28\,LW\Delta.$$

(where LW = ewe liveweight at the start of mating and LWΔ = liveweight change between two weeks before mating and the end of mating).

In 1998 ewe LW was a significant (15%, $P<0.05$) source of variation in ovulation rate in the E+ group and LWΔ was not a significant (<1%) source of variation. The regression equation of LW and LWΔ on ovulation rate (OR) in the E+ group was:

$$OR = 0.091 + 0.0261\,LW + 0.37\,LW\Delta.$$

In 1998 LW was a significant (9%, $P<0.05$) source of variation in number of lambs carried at scanning in the E+ group and variation due to LWΔ was close to significant (6%, $P=0.1$). The regression equation of LW and LWΔ on number of lambs carried at scanning (SC) in the E+ group was:

$$SC = -0.394 + 0.0286\,LW + 1.56\,LW\Delta.$$

Table 3.7. Mean (±SEM) ewe liveweight (WT) (kg) and liveweight change (LWΔ) (g/d) at mating for ewes in the E+ and E- groups that had a single or multiple ovulation (OR), carried 0, 1 or 2 lambs at scanning (SCN) or conceived in the first or second oestrous cycle of mating (CYC).

	Ewe Liveweight at Start of Mating								**Ewe Liveweight Change During Mating**			
	1997				**1998**				**1997**		**1998**	
	No. EWES		**WT**		**No. EWES**		**WT**		**LWΔ**		**LWΔ**	
	E+	**E-**	**E+**	**E-**	**E+**	**E-**	**E+**	**E-**	**E+**	**E-**	**E+**	**E-**
OR												
1	13	15	55.0 ± 1.4[a]	54.6 ± 1.0[a]	16	13	54.8 ± 2.0[a]	62.6 ± 1.8[bcd]	180 ± 100	20 ± 10	100 ± 20	-50 ± 30
2	26	24	54.9 ± 1.0[a]	56.2 ± 0.9[ac]	23	25	61.4 ± 1.5[bc]	66.0 ± 1.4[d]	60 ± 10	20 ± 10	80 ± 20	30 ± 90
3	0	0	N/A	N/A	0	1	0	60 *	N/A	N/A	N/A	-50*
SCN												
0	2	1	56.3 ± 8.7[abc]	58.0 *	2	4	54.5 ± 3.5[a]	68.9 ± 1.6[bc]	0 ± 100[abc]	0*	170 ± 50[abc]	-150 ± 20[a]
1	17	13	55.5 ± 1.0[a]	53.4 ± 0.9[a]	18	18	57.5 ± 2.0[abde]	65.1 ± 1.6[c]	140 ± 70[b]	20 ± 10[abc]	80 ± 20[bc]	-70 ± 20[ac]
2	20	25	54.3 ± 1.2[a]	56.4 ± 0.9[ad]	19	17	61.4 ± 1.8[cd]	64.2 ± 2.0[ce]	70 ± 10[b]	20 ± 10[abc]	110 ± 20[b]	-30 ± 20[ac]
CYC												
1[ST]	31	28	55.5 ± 1.0[a]	55.6 ± 0.9[a]	31	30	59.3 ± 1.6[ab]	64.1 ± 1.5[bc]	90 ± 40[a]	20 ± 10[b]	100 ± 20[a]	-40 ± 20[b]
2[ND]	8	11	52.7 ± 1.3[a]	55.4 ± 1.1[a]	8	9	57.8 ± 2.5[ab]	68.9 ± 1.5[c]	100 ± 10[a]	20 ± 10[b]	80 ± 30[a]	-140 ± 30[c]

Mean liveweight and liveweight change values within a reproductive parameter that do not have a common superscript letter differ significantly ($P<0.05$).

* Values represent data for one animal

3.1.6. Daily milksolid production and cumulative milksolid yield

Daily milksolid production for E+ single-rearing (n=5), E+ twin-rearing (n=6), E- single-rearing (n=7) and E- twin-rearing (n=6) ewes, measured on one day a week for the first 12 weeks of lactation in 1997, are presented in Table 3.8. Daily milksolid production for twin-rearing ewes in the E+ (n=7) and E- (n=7) groups, measured on one day per week on weeks 1, 3 and 5 of lactation in 1998, are presented in Table 3.9.

In 1997 the daily milksolid production from the E+ single-rearing ewes was significantly ($P<0.05$) greater than the E- single-rearing ewes at the week 2 and week 5 sampling but there were no significant differences at any other sampling. The daily milksolid production of the E+ twin-rearing ewes was significantly ($P<0.05$) lower than that of the E- twin-rearing ewes at the week 5 sampling but there was no significant difference at any other sampling. Mean daily milksolid production over the 12 samplings was significantly ($P<0.05$) greater in the twin-rearing ewes than single-rearing ewes in both treatment groups.

There were no significant differences in daily milksolid production between the E+ and E- twin-rearing ewes at week any samplings in 1998.

Cumulative daily milksolid yields for E+ single-rearing, E+ twin-rearing, E- single-rearing and E- twin-rearing ewes during the first 12 weeks of lactation in 1997, are shown in Figure 3.2. Cumulative milksolid yields were significantly ($P<0.001$) greater in the E+ single-rearing ewes than the E- single-rearing ewes, and significantly ($P<0.01$) greater in the E- twin-rearing ewes than in the E+ twin-rearing ewes. Twin-rearing ewes had significantly greater cumulative milksolid yield than single-rearing ewes in both the E+ ($P<0.1$) and E- ($P<0.001$) groups. There were no significant treatment X time interactions on cumulative milksolid yield between any group.

Cumulative daily milksolid yield for twin-rearing ewes in the E+ and E- groups during the first 5 weeks of lactation in 1998, are shown in Figure 3.3. There were no significant differences in cumulative daily milksolid yield between the E+ and E- twin-rearing ewes during the first 5 weeks of lactation in 1998.

Table 3.8. Mean (±SEM) daily milksolid production (g/d) for E+ and E- twin and single-rearing ewes during the first 12 weeks of lactation in 1997.

	TWIN-REARING		SINGLE-REARING	
WEEK	**E+**	**E-**	**E+**	**E-**
1	312±19	294±33	247±36	257±17
2	417±57^{ab}	478±67^{ab}	365±48^{a}	267±20^{b}
3	295±38	345±48	329±37	265±31
4	338±49	354±50	357±32	312±26
5	213±28^{a}	307±41^{b}	219±17^{a}	167±22^{b}
6	311±39	334±51	256±28	260±28
7	218±27	319±56	191±9	191±7
8	184±18	170±38	153±8	165±14
9	164±12	182±46	178±12	156±15
10	127±15	169±51	164±9	168±23
11	136±13	152±43	141±15	120±15
12	164±9	143±29	119±26	126±6

Means with different superscript letters between treatments within a row differ significantly ($P<0.1$)

Table 3.9. Mean (±SEM) daily milksolid production (g/d) for twin-rearing ewes in the E+ and E- groups during weeks 1, 3 and 5 of lactation in 1998

WEEK	**E+**	**E-**
1	343±21	353±36
3	236±16	255±25
5	207±11	194±10

Figure 3.2. Mean (±SEM) cumulative milksolid yield (g) for twin- and single-rearing ewes in the E+ and E- groups during the first 12 weeks of lactation in 1997.

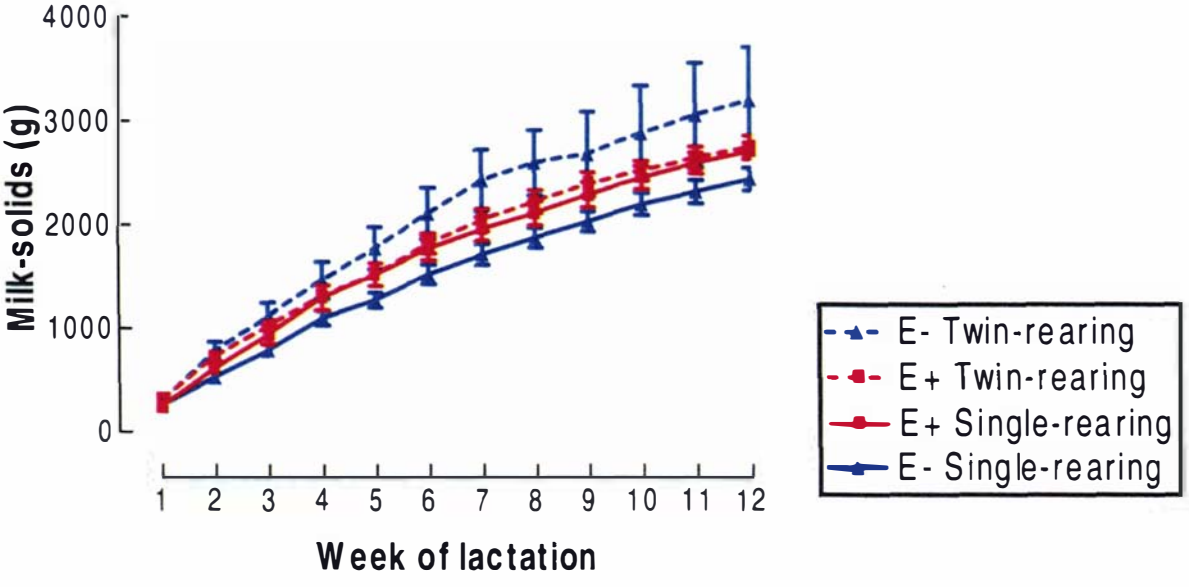
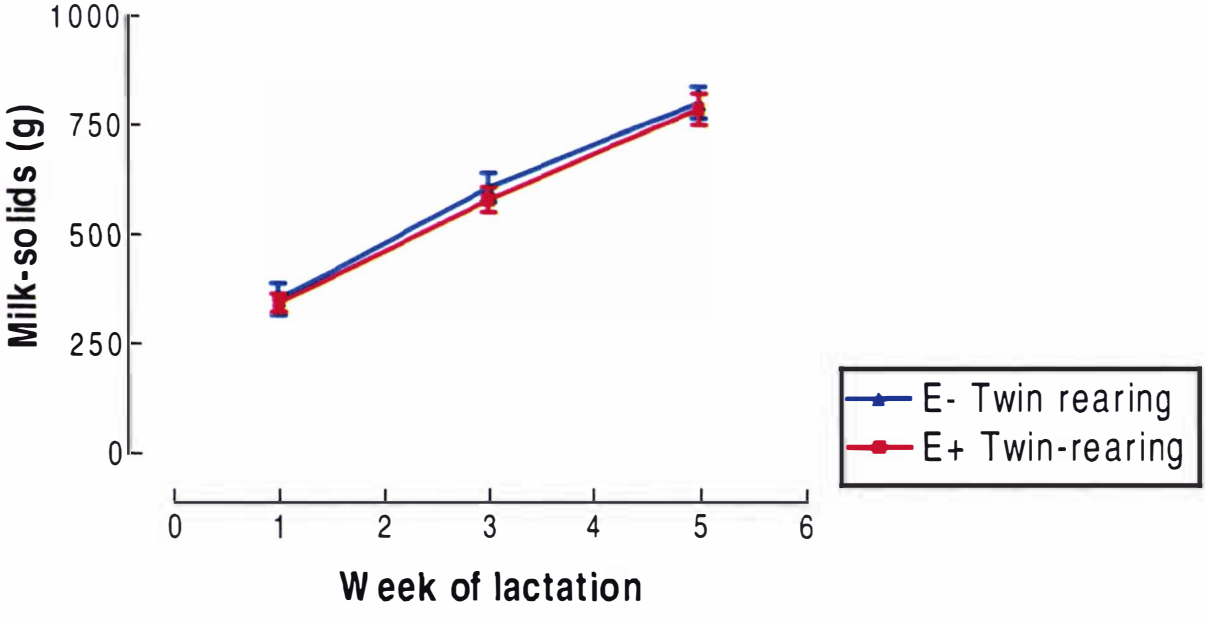


Figure 3.3. Mean (±SEM) cumulative milksolid yield (g) for twin-rearing ewes in the E+ and E- groups during the first 5 weeks of lactation in 1998.



3.1.7. Lamb birthweight

Birth weight data for the twin and single lambs in the E+ and E- groups are presented for both years in Table 3.10.

There were no significant differences in the birth weight of single or twin lambs between the E+ and E- ewes in either 1997 or 1998. Single lambs were significantly ($P<0.05$) heavier than twin lambs in both years.

There was no significant effect of lamb sex on birthweight in either year.

Table 3.10. Mean ($\pm$SEM) birthweights (kg) of twin and single lambs born to ewes grazing either E+ or E- ryegrass.

YEAR	E+ TWINS	E- TWINS	E+ SINGLES	E- SINGLES
1997	4.59 $\pm$ 0.12	4.52 $\pm$ 0.11	6.07 $\pm$ 0.31	5.91 $\pm$ 0.21
1998	4.55 $\pm$ 0.16	4.59 $\pm$ 0.14	5.48 $\pm$ 0.19	5.52 $\pm$ 0.24

3.1.8. Lamb growth rate

Daily lamb growth rates for 1997 and 1998 are shown in Figures 3.4. and 3.5. respectively.

In 1997, there was no significant difference in daily growth rate from birth to six weeks old between twin lambs born on the E+ or E- treatments. Daily growth rate over the six week period was significantly ($P<0.001$) higher in the E+ single lambs (400 $\pm$13 g/h/d) than the E- single lambs (317 $\pm$ 11 g/h/d) (Figure 3.4.). There was no significant effect of time (weeks) or interaction between endophyte and time in any group of lambs. The liveweights of the single lambs at six weeks old were 22.2 $\pm$ 1.0 kg and 18.1 $\pm$ 0.6kg ($P < 0.01$) for the E+ and E- treatments respectively. There was no difference in the liveweights at six weeks old of the twin lambs (15.0 $\pm$ 0.6 kg and 14.4 $\pm$ 0.5 kg for the E+ and E- groups respectively).

In 1998, twin lambs born on the E- treatment had significantly ($P<0.05$) higher daily growth rates during the first three weeks of life than twin lambs born on the E+ treatment (222 $\pm$ 17

g/d vs 139 ± 12 g/d for the E- and E+ treatments respectively) (Figure 3.5.). Growth rates of twin lambs between three weeks of age and eighteen weeks (weaning) were not significantly different between the treatment groups. However, E- twin lambs (33.6 ± 1.2 kg) were significantly ($P<0.05$) heavier at eighteen weeks than E+ twin lambs (30.1 ± 0.7 kg).

Single lambs born on the E- treatment had significantly higher ($P<0.01$) daily growth rates during the first nine weeks of life that the single lambs born on the E+ treatment. During this period the E- single lambs were growing on average 76 ± 11 g/h/d more than the E+ single lambs. There was no significant difference in the daily growth rate of single lambs from weeks nine to eighteen (weaning) between the E+ and E- treatments. However, the E- single lambs (37.8 ± 1.2 kg) were significantly ($P<0.01$) heavier than the E+ single lambs (33.7 ± 1.0 kg) at weaning. There was no significant effect of time (weeks) or interaction between endophyte treatment and time in 1997. In 1998, time (weeks) was a significant ($P<0.01$) effect on lamb growth rate in all groups of lambs. There was no interaction between endophyte treatment and time in 1998.

Figure 3.4. 1997 mean (±SEM) daily growth rates of twin and single lambs born to ewes grazing either E+ or E- ryegrass.

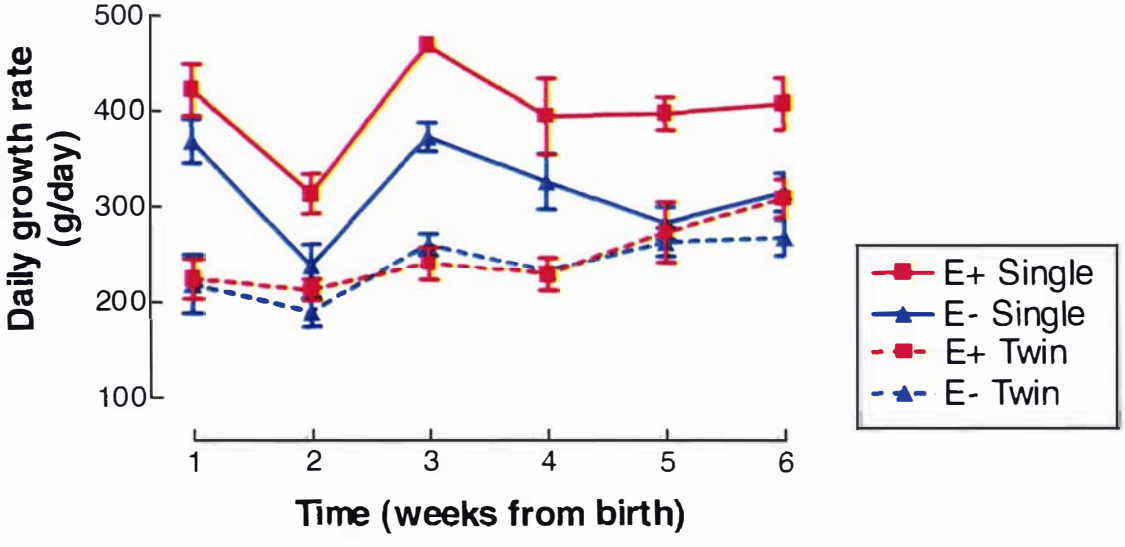
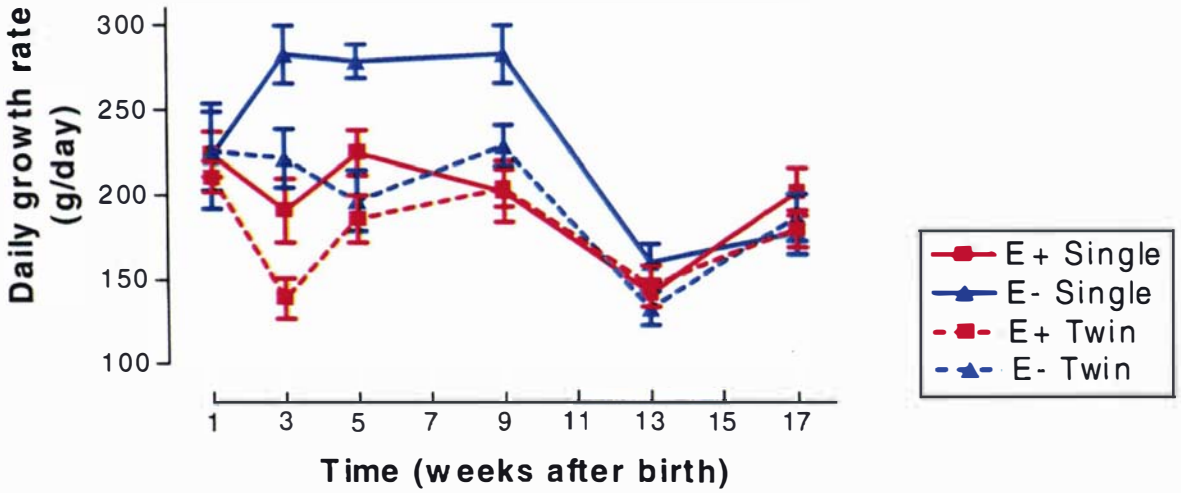


Figure 3.5. 1998 mean (±SEM) daily growth rates of twin and single lambs born to ewes grazing either E+ or E- ryegrass.



3.1.9. Relationship between milk production and lamb growth rate

Regression analysis of daily milksolid production of each ewe at each sampling in 1997 on the daily growth rate of their lamb(s) between samplings failed to show a significant relationship between daily milksolid production and daily growth rate of the E+ twin lambs or any of the E- lambs. Daily milksolid production was a small significant ($P<0.05$) source of variation (11.4%) in the daily growth rate of E+ single lambs.

Regression equation: $dLGR = 0.319 + 0.000255\ MS$

Where dLGR = daily lamb growth rate and MS = daily milksolid production.

There was no significant relationship between daily milksolid production and lamb growth rate in 1998 although the sample size was small and milk production was only measured on 3 occasions.

Regression analysis of cumulative milksolid yield on lamb liveweight showed that the relationship between these two parameters was greater in single than twin lambs and greater in E+ than in E- lambs (Table 3.11.).

Table 3.11. Slope (±SEM) and r^2 for the regression analysis of cumulative milksolid and lamb liveweight for each group in 1997.

	E+ SINGLE	E-SINGLE	E+TWIN	E-TWIN
SLOPE	102 ± 6^a	110 ± 6^a	77 ± 6^b	104 ± 9^a
r2	0.91	0.86	0.84	0.75

Means with different superscript letters between treatments within a row differ significantly ($P<0.05$)

Regression analysis of cumulative milksolid production for each ewe at the final sampling on liveweight of their lambs at the final sampling in 1997 showed no significant positive relationships between the two parameters. r^2=0.37, 0.14, 0.28 and 0.19 for the E+ single, E- single, E+ twin and E- twin groups respectively. When data was pooled across groups there was a significant ($P<0.005$) positive relationship between cumulative milksolid yield and lamb liveweight at the final sampling in 1997. r^2=0.32. There was a significant ($P<0.001$) relationship between cumulative milksolid yield and lamb liveweight at the final sampling in 1998. r^2=0.92 and 0.97 for the E+ and E- groups respectively.

3.1.10. Ewe greasy fleeceweight

The greasy fleeceweights of the E+ and E- groups for 1997 and 1998 are shown in Table 3.11. Ewes grazing E+ pasture had significantly ($P<0.05$) lower greasy fleeceweights than ewes grazing E- pasture in 1998, however, the difference in fleeceweight between the groups in 1997 was not significant. Ewe liveweight was a significant source of variation in fleeceweight and in both the E+ (r^2=0.52) and E- (r^2=0.46) groups.

Table 3.12. Mean (±SEM) greasy fleeceweight (kg) of ewes grazing either E+ or E- ryegrass in each year (1997, 1998).

YEAR	E+	E-
1997	3.72 ± 0.11	3.95 ± 0.13
1998	3.98 ± 0.18^a	4.49 ± 0.14^b

Means with different superscript letters within a row differ significantly ($P<0.05$)

3.1.11. Faecal soiling (dags)

Ewes grazing E+ pasture had significantly (P<0.001) more faecal soiling than ewes grazing E- pasture in autumn 1997 and both summer and autumn 1998 (Table 3.12.).

Table 3.13. Mean (±SEM) faecal soiling scores of E+ and E- ewes during summer and autumn in each year (1997, 1998).

SEASON/YEAR	E+	E-
SUMMER' 97	1.4 ± 0.2	0.8 ± 0.2
AUTUMN' 97	1.9 ± 0.1[a]	0.5 ± 0.1[b]
SUMMER' 98	1.4 ± 0.2[a]	0.6 ± 0.1[b]
AUTUMN' 98	1.7 ± 0.2[a]	0.8 ± 0.2[b]

Means with different superscript letters within a row differ significantly (P < 0.05)

3.1.12. Ryegrass staggers and lethargy

Ryegrass staggers was not observed in the ewes grazing E- pasture at any time during the trial. There were no obvious cases of ryegrass staggers in the E+ ewes during 1997. However, during February and March 1998, 70% of the E+ ewes were affected with ryegrass staggers with 20% of the group being so severely affected that they were unable to be moved.

In addition to the obvious symptoms of ryegrass staggers, ewes in the E+ group were frequently more lethargic than ewes in the E- group. This did not appear to be related to ryegrass staggers as the lethargy was present in animals that did not appear to be suffering from RGS. This lethargy resulted in longer time requirements for moving and any handling of the E+ group.

3.1.13. Body temperature

Mean rectal temperatures of ewes in the E+ and E- groups, recorded during summer/autumn in 1997 and 1998 at a range of ambient temperatures, are presented in Table 3.14.

E+ ewes had significantly (P<0.001) higher rectal temperature than the E- ewes when the ambient temperature at recording was equal to or above 22°C. The average (± SEM) difference in mean rectal temperature above 22°C ambient temperature was 0.55 ± 0.11°C.

Table 3.14. Mean (±SEM) rectal temperature (°C) of E+ and E- ewes at different ambient temperature ranges.

Month/year	Ambient Temp (°C) at recording	E+	E-
Feb' 98	**25.6**	39.90 ± 0.08[a]	39.49 ± 0.06[b]
Feb'97	**24.5**	39.18 ± 0.09[a]	38.60 ± 0.06[b]
Mar'97	**23.8**	40.07 ± 0.04[a]	39.71 ± 0.07[b]
Mar'97	**22.6**	40.47 ± 0.10[a]	39.60 ± 0.08[b]
Apr'98	**21.2**	39.86 ± 0.07	39.85 ± 0.06
Mar'98	**20.2**	39.9 ± 0.09	40.02 ± 0.10

Means with different superscript letters within a row differ significantly (P<0.05)

3.1.14. Urinary zearalenone

There were no significant differences in zearalenone concentration in the urine between the E+ and E- ewes (0.79 ± 0.96 ng/ml and 0.70 ± 0.69 ng/ml respectively) in March 1996.

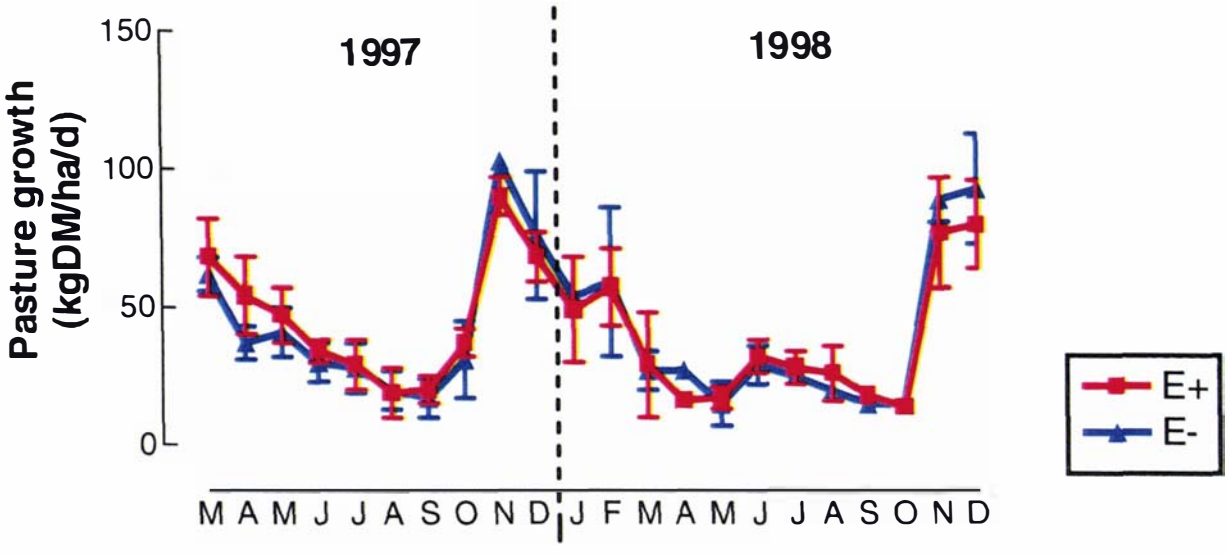
3.2. Pasture measurements

3.2.1. Pasture growth rate

Daily pasture growth rates during each month in 1997 and 1998 are shown in Figure 3.6.
There was no significant difference in daily pasture growth rate between E+ and E- pasture from March 1997 to February 1998. Between March 1998 and December 1998 there was no significant difference in daily pasture growth rate, with the exception of April, when the E- pasture grew significantly (P<0.05) faster than the E+ pasture (27 ± 3 kgDM/ha/d vs 16 ± 3 kgDM/ha/d respectively). There was significant (P<0.01) variation between months in each year, with higher growth rates in the spring to autumn period (October to April) than during

the winter (May to September). There was no significant interaction between months and endophyte status.

Figure 3.6. Mean (±SEM) daily pasture growth rate of E+ and E- ryegrass pastures between March 1997 and December 1998.



3.2.2. Pasture ergovaline and lolitrem B levels

Pasture ergovaline and lolitrem B levels in the ryegrass leaf blade and sheath in both years are shown in Figures 3.7. and 3.8. respectively.

There was a significant ($P<0.001$) effect of season on ergovaline levels in the ryegrass leaf blade (RGLB) and ryegrass leaf sheath (RGLS) components of the E+ pasture. Ergovaline levels were significantly higher during late spring, summer and autumn (November to April) in the RGLB and RGLS than levels in the winter (May to September). This seasonal pattern was similar in both years.

During periods of increased ergovaline production, levels were significantly ($P<0.001$) higher in the RGLS than in the RGLB in both 1997 and 1998. Ergovaline levels in RGLS were often twice the concentration in the RGLB during the summer and autumn. Ergovaline levels were significantly ($P<0.05$) higher in the RGLB and RGLS during mating (March to May) in 1998 than they had been during mating in 1997.

No ergovaline was detected in the E- pasture at any time of the year in either year.

Lolitrem B levels in the E+ pasture followed similar significant (P<0.001) seasonal patterns to ergovaline. However, lolitrem B levels did not increase in the pasture until towards the end of summer (February), unlike ergovaline levels that started to increase in the spring. Lolitrem B levels in the RGLS were generally 3 to 4 times higher than in the RGLB during autumn. Lolitrem B levels were very low or non-detectable during the winter in both RGLB and RGLS. Lolitrem B levels were significantly (P<0.05) higher in the RGLB and RGLS during mating 1998 than during mating 1997.Trace levels of lolitrem B were detected in two samples collected from the E- plots in 1998.

Figure 3.7. Mean (±SEM) ergovaline concentrations in the leaf blade and leaf sheath components of the E+ pasture during 1997 and 1998.

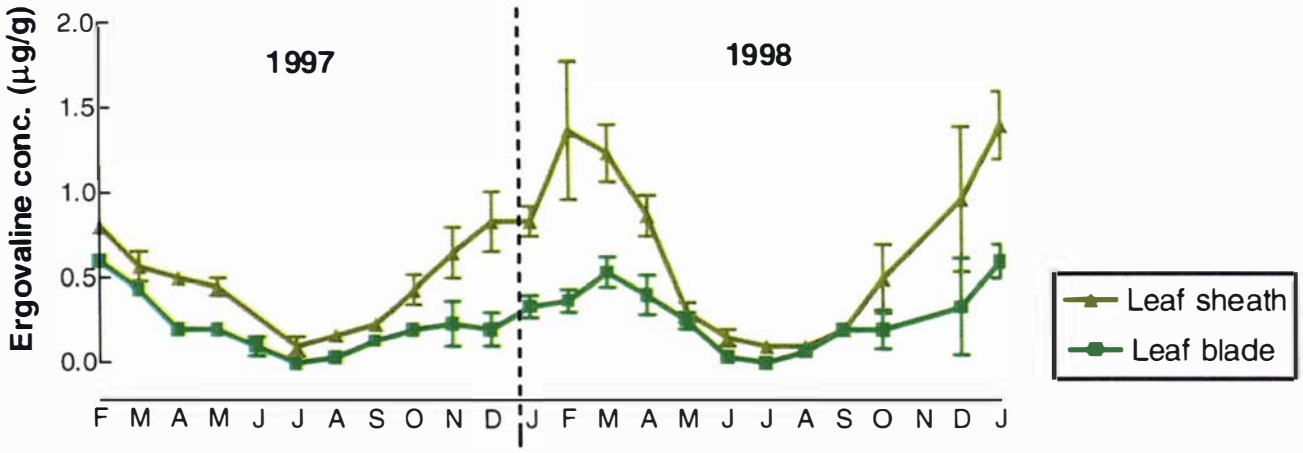
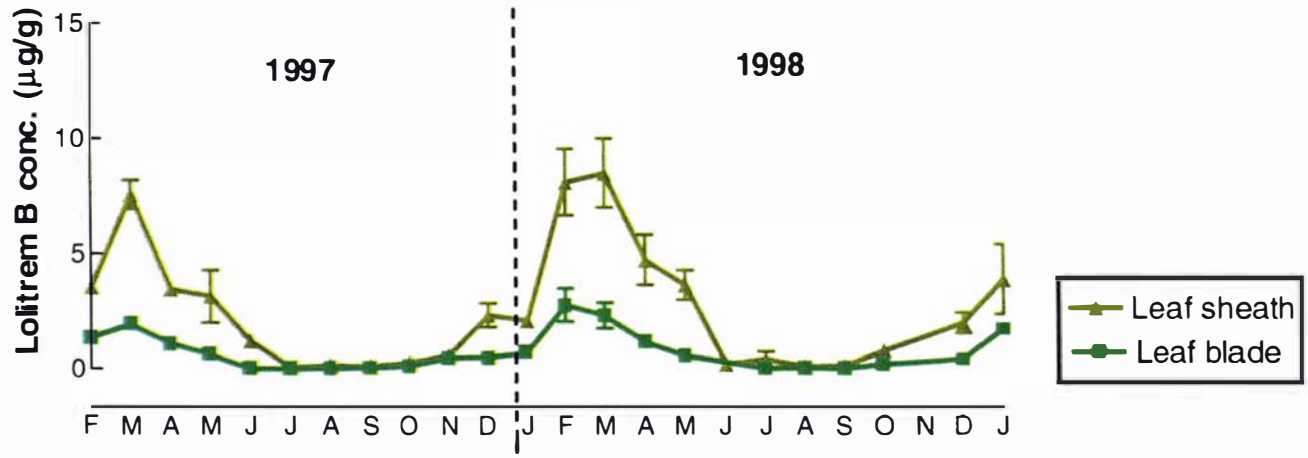


Figure 3.8. Mean (±SEM) lolitrem B concentrations in the leaf blade and leaf sheath components of the E+ pasture during 1997 and 1998.



3.2.3. Endophyte infection levels

In summer 1997 all of the ryegrass tillers examined from the E+ pastures were infected with endophyte, and there were no infected tillers found in the E- pastures. In summer 1998, 98% ± 1% of tillers sampled in the E+ pastures were infected with endophyte and there were no infected tillers found in the E- pastures.

3.2.4. Pasture nutritional analysis

Pasture nutritional components from the E+ and E- pastures are presented in Table 3.14.

There were no significant differences between years or within seasons so the means presented are pooled across years. There was no significant effect of endophyte on any of the nutritional parameters examined. Pasture protein, lipid, ADF, NDF, OMD and ME were all significantly ($P<0.05$) affected by season in the E+ and E- pasture. There was no significant interaction between endophyte and season.

Table 3.15. Mean (±SEM) %protein (PROT), %lipid, %ash, %acid detergent fibre (ADF), %neutral detergent fibre (NDF), %organic matter digestibility (OMD) and metabolisable energy (ME) (MJ) in E+ and E- ryegrass pasture.

	SUMMER		AUTUMN		WINTER		SPRING	
	E+	E-	E+	E-	E+	E-	E+	E-
PROT	13.1 ± 1.9	15.1 ± 0.2	23.6 ± 1.8	23.2 ± 1.9	22.2 ± 0.3	24.8 ± 0.1	20.4 ± 8.9	16.3 ± 7.2
LIPID	2.6 ± 0.3	3.1 ± 0.1	3.9 ± 0.4	3.9 ± 0.4	3.4 ± 0.1	3.6 ± 0.3	3.15 ± 0.5	3.3 ± 0.6
ASH	10.0 ± 0.3	10.7 ± 0.5	12.9 ± 0.4	12.4 ± 0.3	11.9 ± 0.6	12.3 ± 0.4	11.5 ± 1.3	10.8 ± 1.7
ADF	32.9 ± 1.9	30.7 ± 1.2	26.5 ± 3.2	26.0 ± 2.9	25.2 ± 2.7	24.3 ± 3.0	25.9 ± 0.8	27.1 ± 0.7
NDF	53.8 ± 2.0	51.8 ± 1.5	43.9 ± 4.5	43.3 ± 3.6	43.6 ± 2.5	42.0 ± 2.9	44.2 ± 2.5	47.8 ± 2.4
CHO	7.9 ± 0.3	7.8 ± 0.8	7.6 ± 2.1	8.5 ± 1.3	7.9 ± 2.1	8.0 ± 2.2	8.9 ± 3.2	9.4 ± 2.4
OMD	65.1 ± 3.5	70.6 ± 2.1	78.4 ± 3.8	78.9 ± 3.6	78.9 ± 3.8	81.4 ± 2.9	80.7 ± 2.6	78.8 ± 2.6
ME	9.7 ± 0.5	10.5 ± 0.3	11.7 ± 0.6	11.8 ± 0.5	11.8 ± 0.6	12.2 ± 0.5	12.0 ± 0.4	11.8 ± 0.4

3.2.5. Pasture allowance and post-grazing residues

Mean pre-grazing pasture allowance and post-grazing residual of the E+ and E- groups in spring, summer and autumn in both years are presented in Table 3.16.

There were no significant differences in post-grazing herbage mass between the E+ and E- groups during spring and summer in 1997 or 1998. During autumn 1997 and 1998, ewes grazing the E- pasture left significantly ($P<0.001$) lower post-grazing herbage mass than ewes grazing the E+ pasture.

Table 3.16. Mean (±SEM) pre-grazing pasture allowance and post-grazing pasture residues of the E+ and E- groups in spring, summer and autumn in each year (1997, 1998).

	Pasture offered (kg DM/ha)		**Post-grazing residues (kg DM/ha)**			
	1997	**1998**	**1997**		**1998**	
Season			**E+**	**E-**	**E+**	**E-**
Spring	3710 ± 156	3269 ± 213	1228 ± 120	1374 ± 136	1467 ± 93	1704 ± 126
Summer	3859 ± 128	3824 ± 146	2348 ± 145	1959 ± 265	2447 ± 131	2088 ± 237
Autumn	2668 ± 238	2223 ± 153	1425 ± 125[a]	1077 ± 69[b]	1793 ± 115[a]	870 ± 55[b]

Means with different superscript letters within a row differ significantly ($P<0.01$)

4. Discussion and conclusions

4.1. Reproductive performance

This grazing trial was the first attempt to examine the effects of endophyte-infected perennial ryegrass on reproductive parameters such as ovulation rate, number of foetuses carried at

pregnancy scanning and lambing percentage in the ewe. Grazing endophyte-infected perennial ryegrass did not directly affect any of these parameters in any year during the trial period. While there were no significant effects of grazing E+ ryegrass on reproductive performance within any one year of the trail, examination of reproductive performance for the entire pre-trial and trial period, showed that there was a significantly greater proportion of dry ewes in the E+ group compared with the E- group. Much of this difference in dry ewe numbers was due to a high (30%) number of dry ewes in the E+ group in 1999. However, these results indicate that reproductive performance can be reduced in animals grazing E+ ryegrass pastures in long-term grazing situations (3-4 years). It is not likely that the adverse effects will be large enough to be significant in a relatively short-term grazing period.

Grazing endophyte-infected tall fescue has been shown to reduce ovulation rate in ewes (Kramer *et al.*, 1999) however, it would appear the effects of grazing endophyte-infected perennial ryegrass were not as severe. The major factor in this difference in severity between infected tall fescue and ryegrass is likely to be the comparatively lower toxin levels in E+ ryegrass. Mean ergovaline levels in the ryegrass leaf blade during mating in this trial were 0.3 ppm and 0.5 ppm for 1997 and 1998 respectively whereas levels of ergovaline in endophyte-infected tall fescue pastures often exceeded 1 ppm. In addition, E+ tall fescue produces toxins such as lolines that are not produced by E+ ryegrass and these may act additively or synergistically with the other major toxins to exacerbate the toxicoses. However, E+ ryegrass pastures may sometimes produce ergovaline levels above 1 ppm (Lane *et al.*, 1997a) and extreme levels up to 27 ppm have been measured in natural associations of endophyte-infected perennial ryegrass under glasshouse conditions (Lane *et al.*, 1997b). It is possible that ewes grazing these pastures where ergovaline levels exceed 1 ppm would suffer a reduction in fertility.

The published data on the effects of endophyte on ovarian function in grazing ruminants, is small with some evidence that endophyte toxins affect various reproductive hormones that control ovarian function. There is also evidence that endophyte toxins affect gonad function in laboratory animals (Varney *et al.*, 1987; Zavos *et al.*, 1986). This evidence suggests that impaired gonad function may be a factor contributing to reproductive dysfunction in animals grazing endophyte-infected pasture.

There is a large body of evidence on the suppressive effects of the ergopeptine alkaloids on serum prolactin and it is likely that this may in some way disrupt ovarian function as well as other physiological processes associated with reproduction. Treating ewes with bromocriptine to suppress serum prolactin levels has resulted in fewer ovulations than in untreated ewes (Rodway *et al.*, 1983). However, other important hormones such as LH and progesterone may have also been affected by the bromocriptine treatment and caused this reduction in ovulation rate.

At mating there were large variations in serum prolactin levels, largely due to the ewes being at different stages of the oestrous cycle when blood samples were taken. A significant difference in serum prolactin was detected between the E+ and E- ewes at mating in 1997. Analyses of the 1998 blood samples at mating indicated that serum prolactin was suppressed in the E+ ewes, however, large within group variation prevented this difference from reaching statistical significance ($P = 0.15$). Prolactin levels in the cyclic ewe range from as low as 10 ng/ml during the inter-oestrus period to as high as 300 ng/ml during oestrus (Kann, 1971), and as a consequence, there were large variations in serum prolactin level of the ewes sampled in the present study.

None of the other major hormones associated with reproduction were measured in this grazing trial, and are examined in a later trial described in Chapter VI. The effects of endophyte on serum prolactin levels in cyclic ewes are also examined further in Chapter VI.

If, in fact, many of the major hormones associated with reproduction are affected by endophyte toxins, then critical toxin levels at which hormone secretion is sufficiently disrupted to affect fertility need to be determined. It may be that levels of endophyte toxins in endophyte-infected perennial ryegrass pasture are below this critical level as appeared to be the case in this trial.

Mean mating date was significantly ($P < 0.05$) delayed by 1.8 days in the E+ ewes in both 1997 and 1998. This result is consistent with that of Eerens *et al.* (1994) who found that ewes grazing E+ perennial ryegrass had a four-day delay in parturition date compared with E- ewes that was attributed to a delay in mating date rather than an extension of gestation. The underlying mechanisms of this delay in mating date have not been determined. However, it is

possible that the suppression of serum prolactin has a role. Serum prolactin levels have been associated with the control of seasonality in the ewe (Rhind *et al.,* 1980) and mating behaviour in the ram (Howles *et al.,* 1980). It is, therefore, likely that ewes with suppressed prolactin levels as a result of grazing endophyte-infected pasture will have disruptions to their oestrus activity.

Comparatively lower levels of ergovaline in E+ perennial ryegrass compared with E+ tall fescue may be the reason why prolactin was not suppressed to the same extent as in ewes grazing E+ tall fescue. Despite the comparatively lower ergovaline levels in E+ perennial ryegrass, there were several significant effects of endophyte toxicosis exhibited in this trial, many of which could indirectly affect reproductive performance. Why effects of endophyte on thermoregulation, liveweight and faecal moisture should be so highly significant and effects on reproduction not so, is unclear. It is possible that the physiological mechanisms controlling the systems affected are more sensitive to the endophyte toxins than the reproductive system. Alternatively, there could exist a 'threshold' toxin level above, which reproductive loss will occur and the E+ pastures in this trial were below that.

4.2. Ewe liveweight

Lower liveweight in the E+ group of ewes throughout the duration of the trial was the most significant effect of endophyte. The weight difference between the E+ and E- ewes was present at all times during the year but was greater during the spring to autumn period when toxin levels in the E+ pasture were highest.

The depression of liveweight in animals grazing endophyte-infected tall fescue is well documented and there have been several reports describing reduced liveweight in sheep grazing endophyte-infected perennial ryegrass (Fletcher & Barrell, 1984; Fletcher *et al.,* 1999). Although the effect on weight gain is well documented, the mechanisms by which the presence of endophyte in forage precipitates this effect are unclear. There are a few possible mechanisms by which this occurs. Some studies have suggested differences in digestibility between E+ and E- forage. Aldrich *et al.* (1993b) found that the dry matter and organic matter digestibility were 9% lower in E+ tall fescue than E- tall fescue. However, conflicting reports exist that have not found differences in digestibility between E+ and E- tall fescue (Stamm *et*

al., 1994). Pasture nutritional analyses conducted in the present grazing trial also found no differences in any of the nutritional parameters measured between E+ and E- pasture at any time of the year. Therefore, these results eliminate any differences in nutritional aspects between E+ and E- forage as causing the difference in ewe liveweight in this trial.

There have been indications that many endophyte toxins affect the gastrointestinal (GI) system (Smith *et al.,* 1997; Oliver, 1997). The exact effects of endophyte toxins on gut motility and nutrient uptake from the GI tract *in vivo* are currently unknown, and this should not be overlooked as a possible cause of poor weight gains in animals grazing E+ forage.

It has been suggested that the alkaloids produced by endophyte may affect appetite (Oliver, 1997). Two important centres affecting feeding appear to be located in the paraventricular nucleus, with α_2-agonist agents stimulating and serotonergic agonists inhibiting feeding activity (Blundell, 1991). D_2 agonists have also been found to suppress feeding behaviour (Blundell, 1991). Therefore, it is likely that many of the alkaloids produced in E+ pastures, which bind to these three receptor types, will affect feeding behaviour of the grazing animal. It is possible that feeding behaviour, modified in this manner, is in part responsible for depressed feed intake and hence liveweight gains. Additionally, E+ forage appears to be less acceptable to the ewes. There appears to be a particular reluctance to graze into the leaf sheath or pseudostem horizon of the sward. Ewes grazing the E+ pasture during the summer and autumn left more residual dry matter than the E- ewes despite the same pre-grazing allowance and grazing time. This demonstrates the reluctance of the ewes to graze the E+ pasture as closely as the E- pasture particularly when the toxin levels are highest in the summer and autumn. Edwards *et al.* (1993) also noted a reluctance of ewes to graze the pseudostem horizon of E+ ryegrass. The effects of endophyte on feed intake and grazing behaviour are discussed further in Chapter VIII.

The most likely cause of reduced weight gains in the ewes grazing the E+ pasture was a reduction in feed intake. There are several reports that have found cattle and sheep grazing E+ tall fescue have lower dry matter intakes (Jackson *et al.,* 1988; Howard *et al.,* 1992; Chestnut *et al.,* 1992; Aldrich *et al.,* 1993a; 1993b). In 1984 Fletcher & Barrell suggested that reduced growth rate in sheep grazing E+ perennial ryegrass may have been due to reduced herbage intake.

Liveweight is a very important determinant of reproductive performance. There are strong associations between liveweight of ewes at mating and the subsequent number of lambs born e.g. (Morley *et al.*, 1978). At mating in 1997 there was no significant difference in liveweight between the E+ and E- groups. Neither liveweight nor liveweight change between two weeks from the start of mating until the end of mating were significant sources of variation in ovulation rate or number of lambs carried at scanning within either group in 1997. By mating in 1998, significant liveweight differences had developed between the treatment groups. Although there was no significant difference in ovulation rate between the E+ and the E- ewes in 1998, there were differences between the groups in the relationship between ewe liveweight and ovulation rate. Regression analysis of the 1998 ovulation rate data showed that the liveweight of the E+ ewes was a significant source of variation in ovulation rate but that liveweight was not a significant source of variation in the sheep grazing E- pasture. This could be due to significantly higher ewe liveweights in the E- group. The reduction in liveweight in many of the ewes in the E+ group may have limited ovulation rate. The fact that ewes that had single ovulations in this group were significantly lighter than ewes that had multiple ovulations is evidence of this. Conversely, liveweights in the E- group may have been sufficiently high so that liveweight was not a limiting factor in ovulation rate. This suggestion is supported by the fact that there were no significant differences in liveweight between ewes that had either single or multiple ovulations in the E- group. This indicates that endophyte may have indirectly affected ovulation rate in the E+ group via changes in liveweight.
The effects of liveweight on lambs carried at scanning in 1997 and 1998 were similar to those on ovulation rate with a lack of effect in 1997 and differential effects between the E+ and E- groups in 1998.

Liveweight change leading up to and during the mating period was generally not a significant source of variation in any of the reproductive parameters measured. However, the number of lambs carried at scanning in the E- group in 1998 was significantly affected by liveweight change. The E- ewes were losing weight prior to and during mating, which may have reduced the fertilisation rate and increased early prenatal loss in these animals. This could explain the relatively large difference between ovulation rate and scanning percentage in the E- ewes. Chronically lower liveweight may also be responsible for the significantly greater number of dry ewes in the E+ group over the entire trial period.

4.3. Milk production

Due to the labour requirement of milking the ewes, only a small number could be milked each year. However, milk production data collected during 1997 indicated that there were differences in milk production between the E+ and E- groups.

Cumulative milksolid yield was significantly higher in the E+ single-rearing ewes than in the E- single-rearing ewes which was also reflected in lamb growth rates, whereas the opposite was true for the twin-rearing ewes. Cumulative milksolid yield at the final sampling was related to lamb liveweight in 1998 and in 1997 when data was pooled for all ewes and their lambs.

Differences in milk production may be due to differences in ewe condition. There were no significant differences in ewe liveweight between the single-rearing ewes in the E+ and E- group, which eliminates this as a possible reason for the differences in milk production between these ewes. However, the difference in milk production between the twin-rearing ewes in the E+ and E- group is associated with a difference in ewe liveweight. Small group sizes mean that differences in milk production, and their relationship with ewe liveweight between the groups of milked ewes, may not be representative of differences between the two treatment groups as a whole.

In 1998 milksolid production was lower in the E+ twin-rearing ewes than in the E- twin-rearing ewes although this difference was not statistically significant. Additionally the infrequent milking and small number of animals used limit the accuracy of these measurements in estimating milk production.

4.4. Lamb growth rate

The growth rate of single suckling-lambs during the first six weeks of life in 1997 was greater in the E+ than in the E- group and there was no difference in the growth rate of twin lambs. The growth rate of both single and twin suckling-lambs was significantly lower in the E+ group than in the E- group in 1998.

The effects of grazing E+ pasture on the growth rate of suckling-lambs were variable and inconsistent, which was in part due to the small number of lambs in each group. However, data collected on lamb growth rate in 1998 suggested that endophyte does have a negative effect on the growth rate of suckling-lambs. The reasons for this depression in growth rate were unclear but may, in part, be due to lower milk production by the E+ ewes. The 1997 milking data indicated that E+ twin-rearing ewes produced less milk than the E- twin-rearing ewes (see Figure 3.2.). However, the strength of the relationship between milk production and lamb liveweight differed according to the rearing status (i.e. twin vs single) and the treatment. It appeared that this relationship was stronger in single than in twin lambs, and stronger in E+ than in E- lambs. These differences in the milk production/lamb liveweight relationships between the groups of lambs indicate that some factor other than milk production may also responsible for liveweight differences in the lambs. There may also have been differences in the amount of herbage consumed by the E+ and E- lambs during the first six weeks of life. Results to be presented in Chapter VIII of this study show that herbage intakes were significantly lower in the E+ twin and single lambs than in the comparable E- lambs between 14 and 17 weeks of age and it is possible that this difference in herbage intake existed before this. Differences in herbage intake between the groups of lambs could be the 'factor', other than milk production, that causes differences in lamb liveweight between groups. This could also explain differences in the milk production/lamb liveweight relationships between groups of lambs observed in 1997. It has been found that twin lambs have a higher herbage to milk ratio in their diet than single lambs (Geenty *et al.*, 1985), and data in Chapter VIII indicate that herbage intakes are greater in E- lambs than in E+ lambs. Therefore, the weaker relationships between milk production and lamb liveweight in twin and E- lambs are likely to be a reflection of higher herbage intakes and hence a reduction in the contribution of milk towards growth of these lambs compared to single and E+ lambs.

Differences in milk production in 1998 between the E+ and E- twin-rearing ewes were not significant. However, the sampling regime may not have been adequate to show any differences between the groups. Therefore it is difficult to determine whether the differences in lamb growth rate between the E+ and E- lambs were due to milk production differences in the ewes. There was strong relationship between milk production and lamb liveweight in both the E+ and E- twin lambs.

The 1998 results are consistent with those of Eerens *et al.* (1994) who also found that lambs suckling ewes that grazed E+ pasture had lower growth rates than lambs suckling ewes grazing E- pasture.

4.5. Wool production and faecal soiling (dags)

Wool production was greater in the E- ewes, however, this was generally related to body weight and was likely to be due to greater feed intake in the E- ewes.

Faecal soiling was a big problem in the E+ group. This resulted in these ewes requiring a higher labour input due to having to remove the dags more regularly.

Increased faecal soiling in sheep grazing endophyte-infected pasture is well documented (Pownall, 1992; Pownall *et al.*, 1993), however, the reasons for it are not understood.

Greater faecal soiling increases the likelihood of flystrike. Fletcher & Sutherland (1993) found that lambs grazing endophyte-infected pasture had higher faecal soiling and incidence of flystrike than lambs grazing endophyte-free pasture. Increased flystrike reduces the value of wool and pelts, reduces liveweight, increases the labour required for care of the animal and can cause death in severe cases.

Possible mechanisms are the effects of endophyte toxins on gastrointestinal motility, and water reabsorption from the GI tract mediated by prolactin. Prolactin has roles in the control of ion and water transport in many tissues in the mammalian body, which include control of salt and water transport by the intestine (Shennan, 1994). It has also been found that increased endogenous serum prolactin levels produce stimulatory effects on absorption of fluid and NaCl by the rat jejunum (Mainoya, 1975) and that prolactin may be responsible for increased water absorption in the rat colon (Mainoya, 1981). It is, therefore, possible that the depression of serum prolactin by endophyte toxins may adversely affect these processes in the sheep and thereby result in an increase in water content of the faeces and subsequent dag formation.

4.6. Body temperature, ryegrass staggers and lethargy

Increased body temperature and ryegrass staggers were two significant effects of endophyte observed in this grazing trial.

The E+ ewes had body temperatures that were up to 0.5°C higher than the E- ewes when ambient temperatures were above 22°C. This finding is consistent with other reports of body temperature of sheep grazing E+ pastures (Fletcher, 1993; Fletcher & Easton, 1997).

Elevated body temperature in animals grazing E+ pasture is generally associated with suppressed serum prolactin (Fletcher & Easton, 1997). Blood samples were only collected for serum prolactin analysis prior to mating. However, the difference in serum prolactin between the E+ and E- groups in 1997 were associated with a difference in rectal temperature between the groups. The ergopeptine alkaloids have direct vasoconstrictive effects that also precipitate an increase in body temperature. Therefore, an increase in rectal temperature may occur independently of any possible influence of prolactin.

Elevated rectal temperature in ewes grazing the E+ pasture was common during late summer and autumn when ambient temperature, humidity and endophyte toxin levels in the pasture were high. This time of the year is generally associated with mating in many New Zealand ewe flocks. It is possible that elevated body temperature during mating could reduce reproductive performance in these flocks. Embryo survival is significantly reduced in heat stressed ewes (Cockrem & McDonald, 1969; Goerke, 1974). That there was no significant difference in returns to oestrus between the E+ and E- groups indicated no difference in embryonic mortality. Although body temperatures were often significantly higher in the E+ group they may not have amounted to heat stress or been high enough to affect reproductive performance. It is possible that under conditions of higher ambient temperature and humidity that heat stress induced by grazing E+ pasture will reduce embryo survival.

Ryegrass staggers was prevalent in late summer and autumn 1998 but was not evident during the same period in 1997. This is likely to be due to the higher lolitrem B levels in the E+ pasture during 1998. The summer of 1998 was also hot and dry, which reduced pasture growth rate and limited feed supply. This forced the E+ ewes to graze the pasture harder during the

late summer and autumn, consuming more of the pseudostem horizon where lolitrem B levels were very high.

The E+ ewes were generally more lethargic than the E- ewes. This, in conjunction with ryegrass staggers in many animals, made it more difficult to handle these animals and significantly increased the amount of time to carryout stock work. The lethargy in the E+ ewes did not appear to be related to RGS as the condition occurred at times when RGS was not evident and in animals that were not suffering from RGS.

The lethargy may be caused by the serotonergic activity of some endophyte toxins given that serotonin controls sleep and wakefulness in many animals (Whitaker-Azmitia & Peroutka, 1990).

4.7. Zearalenone

When conducting trials on grass-dominant pastures, the oestrogenic mycotoxin zearalenone should be considered as a possible source of reproductive loss in sheep grazing these pastures. Zearalenone levels generally increase in the pasture in the autumn at the same time as some of the major endophyte toxins. It is possible that sufficient zearalenone in the pasture could mask any affects endophyte toxins may have had on reproductive performance. Additionally it is possible that the presence of endophyte may affect zearalenone levels. Kramer (1997) found that zearalenone levels were significantly higher in endophyte-infected ryegrass pasture compared to endophyte-free ryegrass pasture. This may have been due to a greater amount of dead material for the *Fusaria* fungi to colonise in the E+ pasture. Urinary levels measured in the E+ and E- groups in 1996 indicated that there were no differences between the treatment groups and that levels were low. No subsequent zearalenone analyses were conducted so the possible effects of zearalenone on ewe reproductive performance at later times during the trial are unknown.

4.8. Pastures

Monthly pasture growth rates were similar for the E+ and E- ryegrass. However, differences in the grazing behaviour between the ewes grazing each pasture resulted in some differences in pasture characteristics, particularly during the summer and autumn.

The E- ewes grazed their pasture more closely than the E+ ewes. This often resulted in a more open pasture with more bare ground. This allowed the opportunity for many non-ryegrass species to infiltrate the E- pasture. The E+ pasture generally maintained a more dense cover of ryegrass psuedostem that the E+ ewes appeared reluctant to graze.

Differences in grazing behaviour, which appeared to be related to a higher feed intake in the E- ewes, effectively meant that the E- pasture was subjected to a greater grazing pressure. At times the higher grazing intensity of the E- ewes put a strain on the E- pasture supply, particularly during summer and autumn.

Further studies on feed intake and grazing behaviour differences between ewes grazing E+ and E- pastures will be given in Chapter VIII.

Examination of epidermal strips of ryegrass tillers in the E- pasture showed no contamination by E+ plants, however, alkaloid analysis detected trace levels of lolitrem Bin some E- pasture samples that may have indicated some low level contamination. The paddocks used for the trial had previously been in non-grass species for several years, and this would have reduced the possibility of contamination by existing ryegrass seed in the soil. On rare occasions, ewes from the E+ treatment would escape into adjacent E- plots, and possibly transfer E+ seed attached to them or in their faeces. However, this was extremely rare and was unlikely to be a significant source of contamination. Some E+ seed may have been dropped or blown into adjacent E- plots, which is the most likely source of contamination. However, positive alkaloid tests in E- samples were few and only in trace levels, and examination of tiller samples failed to detect any endophyte presence in the E- pasture. Therefore, contamination of the E- pastures by E+ plants was not considered significant.

4.9. Conclusions

Many of the previously reported adverse effects of grazing endophyte-infected pastures, such as reduced liveweight gain, elevated rectal temperature, increased faecal soiling and ryegrass staggers were observed in the E+ group of ewes in this trial.

There was a large reduction in the liveweight of ewes grazing E+ pasture that may have been due to reduced feed intake (as indicated by post-grazing residue measurements).

These effects were generally more prevalent or severe in 1998 than in 1997, which may be due to higher endophyte toxin levels in 1998 and the cumulative effects of grazing endophyte-infected pasture.

A delay in mating date of 1.8 days in the E+ group in 1997 and 1998 was the only reproductive parameter significantly affected by endophyte each year. Long term exposure to E+ ryegrass significantly increased the number of dry ewes in this trial.

It appeared that reproductive performance of the ewes grazing the E+ pasture was more likely to be impaired by poor liveweight rather than any direct effects of endophyte toxins on reproductive mechanisms. However, the potential for E+ ryegrass pastures to produce extreme levels of toxins, higher than in this trial, under certain conditions leaves undetermined whether direct effects are likely to be important. Lifetime reproductive performance may be lower in ewes grazing E+ ryegrass pasture.

The growth rate of twin and single suckling-lambs was reduced in the E+ group in 1998 and this may have been due to reduced milk production by the E+ ewes and differences in herbage intake between the E+ and E- lambs.

CHAPTER IV

Effects of endophyte-infected perennial ryegrass on ewe fertility in a Northland environment

1. Introduction

In New Zealand there are regional differences in the reproductive performance of ewes. Lambing percentage and lamb survival to weaning increase from North to South (Quinlivan & Martin, 1971). In addition, animal health problems associated with mycotoxins, such as facial eczema, are generally more severe in Northland than in other parts of New Zealand. This is most likely due to warmer, more humid conditions that favour proliferation of the causative fungi. It is also possible that this trend in reproductive performance and animal health is due to interactions between the various toxins present and the environmental conditions.

There is strong evidence that high ambient temperature exacerbates many of the toxic effects of endophytic pasture on grazing animals. The vasoconstrictive activity of ergopeptine alkaloids, such as those produced in E+ perennial ryegrass pasture, reduce the ability of animals to dissipate excess heat (Rhodes *et al.,* 1991). Cattle grazing Northland E+ perennial ryegrass pastures commonly suffer from heat stress during the summer and autumn when ambient temperatures are high (Easton *et al.,* 1996). Although high daily temperatures are recorded in the South Island of New Zealand, the night temperatures are generally much lower than those in the North Island. Mean daily minimum temperatures (1969-1998) during summer are 9.1°C and 14.0°C for Southland and Northland respectively (National Institute of Water and Atmospheric Research). Easton *et al.* (1996) suggested that animals may be able to recover during the night in these more southerly regions and thereby avoid heat stress. The effects of any interactions between endophyte toxins and ambient temperature on animal physiology, other than the thermoregulatory system, are unclear and need to be investigated.

There have been other reports where environmental conditions may have interacted with the effects of endophyte toxins. Eerens *et al.* (1994) examined the effects of grazing E+ perennial ryegrass on ewe productivity and found no differences in lamb production between ewes grazing E+ or E- pasture. This grazing trial was conducted in Southland where environmental conditions are much cooler than those in the North are. It is possible that these cooler

conditions may have reduced the effects of endophyte. Additionally, there may be differences in toxin production between regions. Although there is no evidence that ambient temperature affects toxin production by the endophyte (Lane *et al.*, 1997a), the longer growing season and greater risk of water stress on the plant in the North may result in higher toxin production than in the South.

The quandary faced by many of New Zealand's Northland farmers, is that the warm, humid conditions that exacerbate many of the endophyte toxicoses also make it essential for endophyte to be present in pastures to protect ryegrass against the greater pressures of insect pests.

It is, therefore, necessary to examine the effects of endophyte over a range of farms under different environmental conditions to identify any interactions between endophyte and the environment.

The objectives of these grazing trials were 1): to examine the effects on ewe fertility of grazing endophyte-infected perennial ryegrass in a warm Northland environment and 2): to assess these effects in a large commercial ewe flock.

2. Materials and methods

2.1. 1998 Trial

A pilot trial was conducted in 1998 on a commercial sheep farm in Northland. This trial was a preliminary investigation into the effects of grazing endophyte-infected ryegrass pasture on the reproductive performance of ewes in a commercial setting with a view to repeating the trial in 1999. A significant aspect of the 1998 trial was the establishment of an endophyte-free ryegrass pasture under the particular Northland environmental conditions.

2.1.1. Animals

Five hundred mixed-age Coopworth ewes were randomly selected from the commercial Northland ewe flock. The ewes had been grazing E+ ryegrass-dominant pastures prior to the

trial. All the ewes were weighed, ear-tagged, and randomly allocated to either group 1 (n=150) or group 2 (n=350) so that each group was of the same average liveweight.

2.1.2. Treatment pastures

Ten hectares was sown (25 kg/ha) in endophyte-free perennial ryegrass (*cv* Nui) in spring 1997. The existing E+ perennial ryegrass-dominant pastures were used for the E+ treatment. Insecticide (Malathion)-treated baits of wheat were used to protect the E- ryegrass seedlings against predation by black crickets (*Teleogryllus commodus*), which are a common pasture pest in Northland (see Plate 4.1.). This was of limited success with significant populations of crickets (assessed visually) being present during the summer in the E- pasture.

The tiller population in the E- pasture (see Plate 4.2.) was reduced by the crickets, which hindered establishment. The cricket populations did not appear to be as high in the resident E+ pastures and the tiller populations were denser than the E- pasture (see Plate 4.3.).

Wild tall fescue plants that were present in the E- pasture during the 1998 trial were removed prior to the 1999 trial by spot spraying with glyphosate.

2.1.3. Flock management

All ewes were dosed with a controlled release zinc oxide bolus (Time Capsule®) in February 1998 to protect against facial eczema.

On 1 March 1998, group 1 was set-stocked at 15 ewes/ha on the E- treatment pasture, and group 2 remained on E+ pasture, set-stocked at the same stocking rate.

On 1 April 1998, Coopworth rams equipped with harness and crayons were introduced to each group at a ratio of 1:50 (rams:ewes). The ewes were yarded every three days to check the ram's crayon and record ewes that had been mated. All E- ewes that survived during mating (n=148) were examined by laparoscopy three to seven days after 1st mating to determine ovulation rate (see Plate 4.4.) (NB: some ewes were examined <3 days post-mating due to the weekly measurement programme). Laparoscopy was conducted on one day per week during the first oestrous cycle. The number of E- ewes laparoscoped during each week was matched

with the same number of randomly selected E+ ewes that had been mated in the same period. This resulted in the same number of E+ ewes (n=150) being examined by laparoscopy as E- ewes.

Plate 4.1. Black cricket (*T. commodus*) in the Northland E- treatment pasture

Plate 4.2. E- pasture during mating 1998

Plate 4.3. E+ pasture during mating 1998

Plate 4.4. Laparoscopic examination of ewe ovaries to determine ovulation rate

After one oestrous cycle (+21 days from start of mating) the crayon colour was changed and ewes that returned to oestrus were recorded. Rams were removed after the second oestrous cycle (+ 42 days from start of mating).

Due to a shortage of E- pasture the ewes on this treatment were removed at the end of mating and grazed with the E+ group.

All ewes that could be accounted for (n=290 and n=133 for E+ and E- groups respectively) were ultrasound scanned at 90 days of gestation to determine the number of lambs carried per ewe.

2.2. 1999 Trial

The 1999 trial was a modified and improved repeat of the 1998 trial using ewes sourced from the same commercial ewe flock. The establishment of the E- pasture in 1998 allowed a group of ewes to be introduced to this pasture several months before mating and thereby reduce the influence of carryover effects of grazing E+ pasture. The better condition of the E- pasture in 1999 also allowed comparable feeding levels between the E+ and E- groups, and E- ewes were grazed on the E- pastures until scanning. (E- and E+ treatment pastures during mating 1999 are shown in Plates 4.5. and 4.6. respectively).

2.2.1. Animals

In August 1998, one hundred twin-bearing Coopworth ewes were selected from the commercial ewe flock on the basis of similar liveweight and serum gamma-glutamyl transpeptidase (GGT) below 50 ng/ml. The ewes had been grazing E+ ryegrass-dominant pastures prior to the trial. The ewes were divided into two even groups of 50 ewes of the same average liveweight. Group 2 was introduced to the E- treatment pasture on 10 August while group 1 remained on E+ pasture.

These groups of ewes started lambing on 1 September and finished on 11 October 1998. The lambs were weaned in early November 1998 and removed from the treatment pastures. The introduction of these groups in August allowed the ewes on E- pasture eight months exposure to the treatment prior to mating. These groups of ewes are also referred to as 'long exposure' E+ and E- groups.

On 1 December a further four hundred ewes were randomly selected from the commercial ewe-flock, ear-tagged and weighed. One hundred ewes (Group 4) were introduced to the E- treatment and the remaining three hundred ewes (Group 3) remained on E+ pasture. This allowed the E- group four months exposure to the treatment. These groups are also referred to as 'short exposure' E+ and E- groups.

Plate 4.5. E- pasture during mating 1999

Plate 4.6. E+ pasture during mating 1999

2.2.2. Flock management

The management of the E+ and E- long exposure ewes (Groups 1 and 2) prior to introduction of the short exposure groups is described in Chapter V.

All ewes were dosed with a controlled release zinc oxide bolus (Time Capsule®) at the end of February 1999 and were given a second bolus on 13 April 1999 to protect against facial eczema.

The short and long exposure groups were set-stocked together on their respective E+ and E- pastures from 1 December 1998 for the duration of the trial.

On the 1 April 1999 Coopworth rams equipped with harness and crayons were introduced to each group at a ratio of 1:50 (rams:ewes). The same mating recording and laparoscopy procedures used in 1998 were used in 1999. All ewes in the long exposure groups that could be accounted for at mating were examined by laparoscopy (n=27/50 and n=30/50 for the E+ and E- groups respectively). The reasons for ewe losses from the long exposure groups between lambing and mating could not be determined, however, death and escape are the most likely reasons.

All the short exposure E- ewes that could be accounted for at mating (n=83/100) and 150 randomly chosen E+ short exposure ewes were examined by laparoscopy. Ewe losses in the E- group were likely to be due to death and escape.

After one oestrous cycle (21 days from the start of mating) the crayon colour was changed and ewes that returned to oestrus were recorded. Rams were removed after the second oestrous cycle (42 days after the start of mating). The four groups of ewes remained on their respective treatment pastures until pregnancy scanning at 90 days of gestation. Numbers of ewes present at scanning were 27, 28, 235, and 65 for groups 1, 2, 3, and 4 respectively. 18 ewes were removed from group 4 and 2 ewes from group 2 before scanning due to flystrike, which accounts for differences in ewe numbers between mating and scanning.
After scanning all ewes were returned to the commercial flock.

2.3. Sampling

2.3.1. Blood

Blood samples were collected by jugular venipuncture from all ewes at the start of mating (1 April).

Blood samples were used to determine prolactin and GGT concentrations. Serum GGT was measured to assess any impairment of liver function in the ewes due to subclinical facial eczema and thereby examine this as a factor in the reproductive performance of the ewes.

2.3.2. Herbage

Pasture samples were collected from the E+ and E- pastures each year prior to the start of mating, at the end of the first oestrous cycle and at the end of mating. Samples were freeze-dried and a subsample was dissected into ryegrass leaf blade and leaf sheath components. The components were pooled across samplings within treatments and years, and analysed for ergovaline and lolitrem B. In 1999 a subsample from each pasture sample was taken, pooled within treatments, and used for nutritional analysis.

2.4. Prolactin analysis

Serum prolactin levels were determined using a double-antibody ^{125}I radioimmunoassy procedure based on the methods of Van Landegham & Van de Weil (1978) at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand (see Chapter VI, section 2.1.2. for details).

2.5. Herbage ergovaline and lolitrem B analyses

Freeze-dried and ground ryegrass samples were measured for ergovaline and lolitrem B levels by HPLC as described by Barker *et al.* (1993) at the Plant Biochemistry Laboratory, AgResearch Grasslands, Palmerston North.

2.6. Statistical analyses

Statistical analyses were carried out using Graph pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.

Ovulation rate, percent of ewes that returned to oestrous, and number of lambs carried at scanning were analysed by chi-square test. Ewe liveweight, serum GGT and prolactin levels were analysed by one-way ANOVA. Multiple and single regression analyses were conducted to determine sources of variation in ovulation rate and number of lambs carried at scanning due to ewe liveweight, liveweight change and serum GGT.

3. Results

3.1. Reproductive performance

Summaries of ewe reproductive performance for the trials in 1998 and 1999 are shown in Tables 4.1. and 4.2. respectively.

There were no significant differences in ovulation rate, or number of lambs carried at scanning between any group of ewes in any year.

Table 4.1. Mean (±SEM) ovulation rate, number of lambs carried/ewe at scanning and percentage of returns to oestrous in groups of ewes grazing either endophyte-infected or endophyte-free perennial ryegrass in 1998.

	E+	**E-**
Ovulation rate	1.74 ± 0.06	1.66 ± 0.06
Number of lambs carried/ewe	1.78 ± 0.07	1.64 ± 0.07
% returns to oestrus	36	32

Table 4.2. Mean (±SEM) ovulation rate, number of lambs carried/ewe at scanning and percentage of returns to oestrous in groups of ewes grazing either endophyte-infected or endophyte-free perennial ryegrass for a long or short duration in 1999.

	E+ SHORT	E- SHORT	E+ LONG	E- LONG
Ovulation rate	1.78 ± 0.07	1.89 ± 0.09	1.64 ± 0.16	1.67 ± 0.17
Number of lambs carried/ewe	1.47 ± 0.06	1.48 ± 0.11	1.44 ± 0.15	1.38 ± 0.16
% returns to oestrus	27	38	18	32

3.2. Ewe liveweight

1998

In 1998 the E+ ewes put on significantly ($P<0.001$) more weight than E- ewes between introduction to the pasture treatments (1 March) and mating (1 April) (3.8 ± 0.2 kg/ewe and 0.8 ± 0.2 kg/ewe for E+ and E- ewes respectively). Mean (±SEM) ewe liveweight at the start of mating was significantly ($P<0.001$) greater in the E+ group than in the E- group (54.9 ± 0.5 kg vs 52.2 ± 0.2 kg for E+ and E- ewes respectively). The mean ewe liveweights of each group did not change significantly during mating.

1999

In 1999 ewes that had been grazing the E- pasture for a long duration (Group 2) prior to mating were significantly ($P<0.05$) heavier than all other ewes at mating. Ewes that had been grazing the E- pasture for a short duration (Group 4) prior to mating were significantly ($P<0.05$) heavier than all ewes that had been grazing E+ pasture. Mean (± SEM) ewe liveweights at mating were 58.4 ± 1.36 kg, 55.8 ± 0.8 kg, 53.5 ± 1.11 kg and 53.21 ± 0.5 kg for the E- long (E-L), E- short (E-S), E+ long (E+L), and E+ short (E+S) groups respectively.

3.3. Relationship between ewe liveweight, pre-mating liveweight change, and reproductive performance

The mean liveweight and pre-mating liveweight change of ewes of different ovulation rate, number of lambs carried at scanning (scanning rank), and cycle of conception in the E+ and E- groups are summarised for the1998 trial in Table 4.3.; and mean liveweight of ewes of different ovulation rate, scanning rank, and cycle of conception in the long- and short-exposure, E+ and E- groups, are summarised for the 1999 trial in Table 4.4.
(NB: Data presented in tables 4.3 and 4.4. is derived from ewes for which a complete record of liveweight and reproductive parameters was possessed. Ewe liveweight change before mating could not be determined in the 1999 due to the lack of pre-trial individual weight records)

1998

There were no significant differences in liveweight of ewes with the same ovulation rate, scanning rank and cycle of conception between the E+ and E- groups. Ewes that carried 1 lamb at scanning were significantly ($P<0.05$) lighter than ewes that carried 2 lambs at scanning in the E- group. For the E+ ewes, the difference was in the same direction, but not statistically significant.

There was no significant differences in pre-mating liveweight change between ewes of different ovulation rate, scanning rank or cycle of conception within a treatment group. Generally E+ ewes put on significantly ($P<0.05$) more weight before mating than E- ewes.

Multiple regression analysis of ewe liveweight and liveweight change on ovulation rate for the E+ group showed that an insignificant amount of the variation in ovulation rate was due to liveweight or liveweight change. Multiple regression analysis of the E- group data showed that liveweight (3.36%) and liveweight change (2.22%) were not significant sources of variation in ovulation rate individually, however, together they were a small significant (5.58%, $P=0.027$) source of variation.

Multiple regression equation of ewe liveweight and liveweight change on ovulation rate in the E- group:

$OR_{(E-)} = 0.593 + 0.0199LW + 0.753LW\Delta$

Where OR = ovulation rate, LW = liveweight and LWΔ = liveweight change.

Multiple regression analysis of ewe liveweight and liveweight change on the number of lambs carried at scanning in the E+ group showed that liveweight was a small significant (3.79%, P=0.041), and liveweight change was not a significant (<1%) source of variation in the number of lambs carried at scanning. Multiple regression analysis on the same parameters in the E- group showed that liveweight was a significant (9.03%, P=0.002), and liveweight change was not a significant (<1%) source of variation in the number of lambs carried at scanning.

Multiple regression equations of ewe liveweight and liveweight change on number of lambs carried at scanning in the E+ and E- groups:

$SCN_{(E+)} = 0.390 + 0.0237LW + 0.542LW\Delta$

$SCN_{(E-)} = -0.406 + 0.0386LW + 0.479LW\Delta$

Where SCN = number of lambs carried at scanning, LW = liveweight and LWΔ = liveweight change.

Ewe liveweight at mating was a greater source of variation in ovulation rate and number of lambs carried at scanning in the E- group than in the E+ group. This was despite what appeared to be similar liveweights at mating between the E+ and E- ewes and similar proportions of ewes in each ovulation rate and scanning rank group.

1999

Ewe liveweights at mating were significantly lower in the E+ groups than in the E- groups. However, when ewes were divided into groups of different ovulation rate and scanning rank within treatment groups there were no longer significant differences in liveweight between E+ and E- with the exception of ewes that carried twins at scanning in the E+ and E- short exposure groups. There were no significant differences in liveweight between ewes of different ovulation rate or scanning rank within a group.

There were no significant differences in liveweight between ewes that conceived in different cycles within or between groups.

Regression analysis of ewe liveweight on ovulation rate and number of lambs carried at scanning showed that liveweight was not a significant source of variation in either ovulation rate or number of lambs carried at scanning in any treatment group.

Table 4.3. Mean (±SEM) liveweight (WT), pre-mating liveweight change (LWΔ) and numbers of ewes of different ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) within the E+ and E- groups in the1998 trial.

	No. EWES		WT(kg)		LWΔ(g/d)	
	E+	E-	E+	E-	E+	E-
OR						
1	59	66	50.7±0.7	50.1±0.7	231±20^{bc}	28±21^{a}
2	63	56	50.9±0.6	52.7±0.8	265±20^{c}	74±13^{a}
3	19	15	51.3±1.5	49.8±1.0	211±33^{bc}	117±30^{ab}
4	-	2	-	59.0±9.5*	-	50±68*
Total	141	139	-	-	-	-
SCN						
0	11	8	50.8±1.4^{ab}	49.9±1.5^{ab}	188±86^{a}	-3±21^{a}
1	39	31	49.6±0.9^{ab}	48.8±0.9^{a}	242±24^{bc}	52±22^{a}
2	65	74	51.2±0.7^{ab}	52.6±0.7^{b}	230±16^{b}	63±20^{a}
3	12	20	52.3±0.9^{ab}	52.5±2.1^{ab}	317±39^{b}	85±48^{ac}
Total	127	133	-	-	-	-
CYC						
1	82	75	51.0±0.6	51.3±0.7	228±15^{b}	65±17^{a}
2	44	42	50.9±0.8	51.4±0.9	283±24^{b}	58±25^{a}
Total	126	117	-	-	-	-

NB: Means with different superscript letters within a reproductive parameter differ significantly ($P<0.05$).

* = data were excluded from analysis due to small numbers of animals.

Table 4.4. Numbers and liveweight (WT) of ewes in each group of different mean (±SEM) ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) within the short- and long-exposure, E+ and E- groups in the1999 trial.

	No. EWES				WT(kg)			
	LONG		SHORT		LONG		SHORT	
	E+	E-	E+	E-	E+	E-	E+	E-
OR								
1	10	12	42	17	54.7 ±1.7	58.0 ±1.9	52.5 ±1.0	54.7 ±1.4
2	12	9	67	29	52.4 ±1.8	57.7 ±2.2	53.5 ±0.7	55.5 ±1.1
3	1	2	21	6	52*	68.5 ±7.5	55.6 ±1.3	59.3 ±1.5
Total	23	23	130	52	-	-	-	-
SCN								
0	5	4	18	14	51.6 $\pm2.4^{a}$	53.9 $\pm3.2^{ab}$	52.5 $\pm1.6^{ab}$	56.4 $\pm1.5^{ab}$
1	3	3	30	10	55.2 $\pm2.1^{ab}$	55.7 $\pm4.6^{ab}$	52.9 $\pm1.4^{ab}$	57.1 $\pm1.0^{ab}$
2	12	13	74	15	51.9 $\pm0.8^{a}$	61.0 $\pm1.9^{b}$	53.8 $\pm0.6^{ab}$	54.4 $\pm1.7^{ab}$
3	3	3	6	3	52.0 $\pm3.3^{ab}$	58.8 $\pm5.2^{ab}$	57.0 $\pm1.9^{ab}$	50.5 $\pm2.0^{ab}$
Total	23	23	128	42	-	-	-	-
CYC								
1	14	10	87	24	55.4 ±2.5	58.6 ±1.6	53.8 ±0.5	55.7 ±1.1
2	9	10	31	15	52.3 ±2.4	61.6 ±2.7	52.0 ±1.0	55.2 ±1.7
Total	23	20	118	39	-	-	-	-

NB: Means with different superscript letters within a reproductive parameter differ significantly ($P<0.05$).

* = data were excluded from analysis due to small numbers of animals.

3.4. Serum prolactin

There was no significant difference in serum prolactin levels between the E+ and E- ewes during mating in 1998. Serum prolactin levels were 54 ± 10 ng/ml (n=50) and 55 ± 13 ng/ml (n=50) for the E+ and E- groups respectively.

Serum prolactin in the E+ ewes was significantly ($P<0.01$) lower than for E- ewes in the short exposure group. There was no significant difference in serum prolactin between the E- long exposure group and the E+ ewes or the E- short exposure group. Serum prolactin levels were 56 ± 8 ng/ml (n=50), 89 ± 24 ng/ml (n=25) and 123 ± 17 ng/ml (n=56) for the E+, E- long and E- short exposure groups respectively. (NB: serum prolactin levels reported for the E+ ewes included both the long and short exposure E+ ewes).

3.5. Serum GGT

1998

Serum GGT levels in the ewes were not significantly different between groups in 1998. Mean (±SEM) serum GGT levels were 120 ± 14 iu/l and 123 ± 14 iu/l for E+ and E- ewes respectively. These levels are consistent with mild sporidesmin toxicity.

1999

There were no significant differences in serum GGT levels between any group of ewes in 1999. Mean (±SEM) serum GGT levels were 58 ± 3 iu/l, 52 ± 2 iu/l, 65 ± 10 iu/l and 56 ± 2 iu/l for the E+S, E-S, E+L and E-L groups respectively. These GGT levels are regarded as not clinically significant.

3.6. Relationships between serum GGT and reproductive performance

Mean serum GGT levels of ewes of different ovulation rate, number of lambs carried at scanning (scanning rank) and cycle of conception in the E+ and E- groups are summarised for the1998 and 1999 trials in Tables 4.5.and 4.6. respectively. (NB: data presented are derived from ewes for which a complete record of GGT and reproductive records was possessed)

1998

There were no significant differences in serum GGT between ewes with different ovulation rates, scanning rank, or cycle of conception within or between treatment groups.

Regression analysis of serum GGT on ovulation rate within the E+ and E- groups showed that serum GGT was not a significant source of variation in ovulation in the E+ group but was a small significant (3.1%, P=0.04) source of variation in the E- group.

Regression equation for serum GGT on ovulation rate in the E- group:

$OR_{(E-)} = 1.76 - 0.000804GT$

Where OR = ovulation rate and GT = serum GGT levels.

GGT level was not a significant source of variation in number of lambs carried at scanning in any group of ewes.

1999

There were no significant differences in serum GGT between ewes with different ovulation rates, scanning rank or cycle of conception within or between treatment groups.

GGT levels were not a significant source of variation in ovulation rate or number of lambs carried at scanning in any group of ewes.

Table 4.5. Mean (±SEM) serum GGT (iu/ml) levels of ewes of different ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) for the E+ and E- groups in the 1998 trial.

	No. EWES		SERUM GGT	
	E+	**E-**	**E+**	**E-**
OR				
1	50	64	128±22	136±23
2	60	55	119±23	119±20
3	18	15	107±29	122±44
4	1	2	56*	148±118*
SCN				
0	7	12	99±26	134±42
1	28	40	194±43	127±26
2	70	69	104±18	119±20
3	20	11	94±19	179±73
4	-	1	-	122*
CYC				
1	77	88	141±23	120±17
2	41	33	92±13	145±37

* = data were excluded from analysis due to small numbers of animals.

Table 4.6. Mean (±SEM) serum GGT levels (iu/ml) of ewes of different ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) for the short- and long-exposure, E+ and E- groups in the 1999 trial.

	No. EWES				SERUM GGT			
	LONG		SHORT		LONG		SHORT	
	E+	E-	E+	E-	E+	E-	E+	E-
OR								
1	10	11	40	15	57±4	56±2	55±2	59±5
2	12	10	61	27	55±3	50±4	54±2	57±3
3	1	2	21	6	50*	44±7	71±17	47±4
4	-	-	-	1	-	-	-	56*
SCN								
0	5	5	15	13	56±2	50±4	58±3	55±3
1	3	3	28	9	57±3	50±5	57±3	58±6
2	12	12	71	14	58±4	51±2	58±5	59±5
3	3	3	6	3	59±4	58±11	52±4	53±5
CYC								
1	14	11	88	21	52±4	51±3	59±5	53±3
2	9	10	29	14	54±3	53±3	54±3	63±5

*= data were excluded from analysis due to small numbers of animals.

3.7. Herbage lolitrem B and ergovaline concentration

1998

Mean (±SEM) herbage lolitrem B concentrations in the E+ pasture during mating 1998 were 2.9 ± 0.2 ppm in the ryegrass leaf blade (RGLB) and 10.0 ± 0.1 ppm in the ryegrass leaf sheath (RGLS). No lolitrem B was detected in the E- herbage during mating 1998.

Mean (±SEM) ergovaline concentrations in the E+ herbage during mating 1998 were 0.8 ± 0.1 ppm and 1.4 ± 0.2 ppm for the RGLB and RGLS respectively. No ergovaline was detected in the E- ryegrass during mating in 1998.

1999

Mean (±SEM) herbage lolitrem B concentrations in the E+ pasture during mating 1998 were 2.8 ± 1.0 ppm and 7.1 ± 1.6 ppm in the RGLB and RGLS respectively. No lolitrem B was detected in the E- pasture during mating 1999.

Mean (±SEM) ergovaline concentrations in the E+ herbage during mating 1998 were 0.6 ± 0.1 ppm and 1.5 ± 0.1 ppm for the RGLB and RGLS respectively. No ergovaline was detected in the E- pasture during mating in 1999.

There was no significant difference in ergovaline concentration between years. Lolitrem B concentrations were higher in the E+ RGLS in 1998 than in 1999 but were not significantly different in the E+ RGLB.

3.8. Pasture nutritional analysis

Pasture nutritional analyses for mating 1999 are summarised in Table 4.7.

There was no significant difference in any nutritional parameter measured between E+ and E- pasture during mating in 1999.

Table 4.7. Mean (±SEM) % protein (PRO), % lipid (LIP), % ASH, % acid detergent fibre (ADF), % neutral detergent fibre (NDF), % carbohydrate (CHO), % organic matter digestibility (OMD) and metabolisable energy (ME) (MJ/kgDM) in the E+ and E- pasture during mating in 1999.

PARAMETER	PASTURE	
	E+	E-
PRO	21.8±0.4	21.4±0.5
LIP	4.0±0.7	4.1±0.6
ASH	13.4±0.1	12.6±0.4
ADF	29.6±1.5	28.2±3.1
NDF	48.4±0.7	47.0±0.7
CHO	5.6±0.3	7.3±0.4
OMD	75.2±3.2	76.8±4.1
ME	11.2±0.5	11.4±0.6

4. Discussion and conclusions

4.1. Reproductive performance and trial limitations

There were no significant differences in reproductive performance between any group of ewes in either year.

Problems were experienced with the relatively poor control over experimental conditions associated with the large area and numbers of animals used, and the isolated location of the trials. Many of these problems are likely to have confounded the reproductive results.

In 1998 the E- pasture had been newly established and was subject to insect attack and a dry summer environment. This resulted in a relatively low dry matter production from the E- pasture compared with the well established E+ pastures. This restricted the time that the E- ewes could be introduced to the E- pasture to 1 month prior to mating. This 1 month period may not have been sufficient time for any carryover effects of grazing E+ pasture on ewe reproductive performance to be reduced. Additionally, low dry matter production by the E-

pasture restricted the liveweight gain of the E- ewes prior to mating in comparison to the E+ ewes. Differences in ewe liveweight, liveweight change and their relationship with reproductive performance are discussed below.

In 1999 many of the problems associated with the E- pasture had been overcome and comparable pasture allowances were possible between treatment groups. It was also possible to introduce a group of 50 ewes to graze the experimental pastures for an 8 month pre-mating period with a second group of 100 ewes being introduced 4 months prior to mating. The objectives of this staggered introduction of ewe groups were to allow longer periods on E- pasture to reduce the possible carryover effects of grazing E+ pasture. Despite a likely reduction in carryover effects, it is also possible that the ewes used in these trials may have suffered from permanent effects of being raised on E+ pastures. These possible permanent effects should not be ignored as significant factors in reduced reproductive performance, however, they could not be quantified in these trials.

Despite better control over feed allowance and a longer trial duration, there were problems associated with maintaining comparable ewe numbers in each group. Due to a relatively large trial area with limited subdivision, and infrequent monitoring of the treatment groups, many ewes could not be accounted for. To compound this problem, some animal health problems resulted in the exclusion of a number of ewes for certain reproductive measurements. The largest of these problems was the high incidence of flystrike experienced in the E- groups towards the end of mating in 1999. This resulted in 22 ewes being excluded from the E- groups at pregnancy scanning. A reasonable explanation for the increased incidence of flystrike in the E- group cannot be offered.

Many of the problems discussed above would have been eliminated in a replicated trial. However, the area and numbers of ewes required in each group and the lack of intensive monitoring meant that replication was not possible in these trials.

These trials highlight the difficulties in reproducing results obtained in relatively small, replicated, strictly controlled grazing trials, in a large, commercial farm situation.

4.2. Ewe liveweight, liveweight change, and their relationship with reproductive performance

In 1998, E- ewes gained significantly less weight prior to mating than E+ ewes and as a consequence were significantly lighter at the start of mating. The poor weight gain of E- ewes was largely due to poorer dry matter production by the E- pasture during the summer and early autumn, which was associated with the problems experienced establishing the pasture. Differences between the E+ and E- pastures are discussed later in section 4.4.

The differences in liveweight and liveweight change between the E+ and E- groups may have confounded any effects of the treatments on reproductive performance. The liveweight differences between the treatment groups appeared to have differential effects on reproductive performance. Multiple regression analyses showed that pre-mating liveweight in the E- group was a significant source of variation in ovulation rate and the number of lambs carried at scanning. The same analysis in the E+ groups showed that liveweight was not a significant source of variation in any of the reproductive parameters measured. This suggests that the poorer liveweight in the E- group may have adversely affected reproductive performance.

In 1999, pasture allowance was similar for each group. Despite this, the E+ groups were significantly lighter at the start of mating than the E- groups. This result is similar to that observed in the small grazing trial described in Chapter III, and has been reported in other studies (Fletcher *et al.*, 1999).

Pre-mating liveweight change could not be determined due to the lack of individual pre-trial weight records. However, it could be assumed that the E- ewes gained more weight during the pre-mating trial period than the E+ ewes as both groups were the same average liveweight before the trial started. However, interpretation of the pre-mating liveweight data is made difficult by ewe losses prior to mating. It cannot be assumed that the missing ewes were a random assortment of weights and, therefore, they may have biased the groups' means. Larger group sizes may have reduced the risk of this. Generally ewe liveweights were significantly better in 1999 than they had been in 1998 across all treatment groups. Regression analysis showed that pre-mating liveweight was not a significant source of variation in reproductive performance in any group of ewes in 1999.

As with reproductive performance, the examination of the effects of grazing E+ pasture on liveweight were made difficult by the large, poorly monitored nature of these on farm trials. However, it is likely that the lower pre-mating liveweights observed in the E+ group were due to the effects of endophyte, despite the possible confounding effects of ewe losses.

4.3. Serum prolactin

In 1998, there was no significant difference in serum prolactin between the E+ and E- ewes during mating. Mean prolactin levels were generally low in both groups of ewes compared with prolactin levels measured in other ewe flocks at this time of the year (See Chapter III section 3.1.2.).

Sheep grazing E+ pasture during the summer and autumn when ambient temperatures are higher than 22°C frequently have lower serum prolactin levels than sheep grazing E- pasture (Fletcher *et al.*, 1999).

Despite high ambient temperature during the mating period and significant quantities of ergovaline present in the E+ pasture there were no differences in prolactin. There are three likely reasons why no difference was detected. Firstly, there were large within group variations in prolactin associated with ewes being at different stages of the oestrous cycle. Where suppression of prolactin by endophyte toxins is relatively small, this variation can mask the effect. The problems associated with measuring differences in prolactin in cyclic ewes were also encountered in the Manawatu trial in 1998. Significant differences in prolactin have been measured in E+ and E- ewes where the oestrous cycles were synchronised (Chapter VI). This showed that, when the variation in prolactin due to cyclic activity was removed, differences due to endophyte could be determined.

Secondly, the difference in plane of nutrition between the E+ and E- ewes due to poor E- pasture availability may have affected serum prolactin levels. Finally, volunteer tall fescue plants were present in some areas of the E- pasture during mating. These plants were likely to be infected with the wild-type endophyte *N. coenophialum*, which is capable of producing ergovaline in greater quantities than E+ ryegrass. Normally the ewes would have avoided these toxic plants in favour of the toxin-free E- ryegrass, however, the reduced availability of the E- pasture may have resulted in ewes consuming some tall fescue. There was visual

evidence that the E- ewes were grazing the tall fescue plants (see Plate 4.7.). Therefore some E- ewes may have been ingesting sufficient quantities of ergovaline to suppress their prolactin secretion.

In 1999, serum prolactin in the E+ ewes was significantly lower than in the E- short exposure ewes and was lower than the E- long exposure ewes although this was not significant. Different cyclic stages were again a significant source of variation in serum prolactin levels. However, the differences in serum prolactin between the E+ and E- short exposure groups were significant despite this variation. Prolactin levels in the E- long exposure group were difficult to interpret due to the relatively small number of animals available for sampling.

Prolactin levels in the E+ ewes during mating in 1999 were similar to levels in 1998, whereas the prolactin levels in the E- ewes were significantly higher in 1999 than they had been in 1998. This may reflect the better nutrition and removal of tall fescue from the E- pasture.

Plate 4.7. Grazed tall fescue plants in the E- pasture in 1998

4.4. Serum GGT

Liver damage caused by sporidesmin has the potential to severely reduce reproductive performance (Smeaton *et al.*, 1985). It was important in this trial that this risk was minimised by protection of the ewes with zinc oxide.

Despite the administration of a single intraruminal zinc oxide bolus to each ewe in 1998, there was mild liver damage in both groups due to sporidesmin toxicity. This liver damage was not severe enough to reduce reproductive performance in the E+ ewes, however, there was a significant inverse relationship between ovulation rate and serum GGT levels in the E- ewes. Despite similar GGT levels in both groups, the poor liveweight performance of the E- ewes may have resulted in a greater susceptibility to the effects of the liver damage on reproductive performance.

Serum GGT levels were significantly lower across all treatment groups in 1999 than they had been in 1998 and were regarded as not being clinically significant. Consequently, liver damage was not considered to be a factor in reproductive performance of the ewes. Additionally, this could have contributed to better ewe liveweights across all groups in 1999.

Lower GGT levels in 1999 could be due to the use of two consecutive zinc oxide bolus treatments that conferred a longer protection period during the late summer and autumn. In addition, outbreaks of clinical facial eczema were not as prevalent in 1999 in the unprotected ewes that were grazing similar pastures on the farm, as they had been in 1998. This suggests that pasture sporidesmin levels may have been lower in 1999 on the farm used for this trial.

4.5. Pastures and insect pests

Difficulty in establishing an endophyte-free pasture in the Northland environment was a major constraint in the 1998 trial. Problems establishing and maintaining E- pastures are often associated with attack from insect pests (Prestige *et al.*, 1985). Additionally, high summer temperatures and moisture stress during establishment will adversely affect the ability of the ryegrass plant to compensate for tiller loss due to insect attack (Hume *et al.*, 1993).
Large populations of black field crickets (*T. commodus*) appeared to be present in the E- pasture and are likely to be a major cause of the poor establishment. These large populations

were present despite treatment with insecticide. This may have been due to the warm, dry spring and summer period being favourable for the insects' proliferation.

The black field cricket is a common pasture pest in Northland (Blank *et al.,* 1986; Blank & Chapman, 1985; Hartley *et al.,* 1982) and outbreaks are often associated with droughts (Blank & Chapman, 1985). The black field cricket is also known to attack endophyte-infected ryegrasses (Blank & Olson, 1987) however, it is possible that the infected grasses have some resistance to the cricket attack. E+ perennial ryegrass has been found to have resistance against other important insect pasture pests. These include Argentine stem weevil (*Listronotus bonariensis*), Pasture mealybug (*Balanococcus poae*), Cutworm (*Graphania mutans*) and Black beetle (*Heteronychus arator*) (Prestidge *et al.,* 1994) as well as up to 40 other insect pests (Prestige & Ball 1996). There is also evidence that black field crickets are adversely affected by endophyte (Quigley *et al.,* 1993: Van Heeswijck & McDonald, 1992), although this has not been confirmed for New Zealand populations.

Therefore, E- ryegrass that does not have the endophyte toxins that confer resistance against insect attack is at considerable disadvantage, particularly in environments such as in Northland. The poor persistence of E- pastures in these environments is the major reason why farmers often must persist with the use of endophyte-infected grasses despite the associated animal health problems.

The poor establishment of the E- pasture also allowed toxic wild tall fescue plants to contaminate the pasture. In 1999, a thicker E- sward had been established and insect pests did not appear as prevalent as they had been in 1998. Additionally the tall fescue plants were removed to eliminate any confounding effects of the toxins produced by these plants.

4.6. Conclusions

There were no significant differences in reproductive performance between ewes grazing E+ and E- ryegrass pastures. However, the large scale, unreplicated design and infrequent monitoring of these trials associated with 'on farm' nature made it difficult to accurately measure differences in reproductive performance. Establishment of an E- pasture was a major problem, which compounded many of the other design limitations.

When the pastures were comparable, the ewes grazing the E+ pasture had significantly lower liveweights and serum prolactin levels than the ewes grazing the E- pasture. These observations are consistent with previously documented effects of grazing E+ pasture. However, interpretation of these results was made difficult due to unaccounted ewe losses.

These trials highlighted the difficulty in reproducing results generated under small intensive trial conditions in a large 'on farm' trial. The difficulty in establishing E- pastures in the Northland was also demonstrated.

CHAPTER V

Ewe liveweight, lamb birthweight, and liveweight gain during lactation on a Northland endophyte-infected ryegrass pasture.

1. Introduction

It has been well established that poor growth rates in lambs from birth to weaning delay puberty and reduce subsequent reproductive performance in both ewes and rams (Dyrmundsson, 1973; Keane, 1974; Hawker, 1977).

There is strong evidence that ewes and lambs grazing endophyte-infected ryegrass have reduced liveweight gains (Fletcher & Barrell, 1984; Fletcher, 1993). Similarly, poor weight gains have been reported in sheep grazing endophyte-infected tall fescue (Debessai *et al.*, 1993).

Therefore, poor weight gain associated with grazing endophyte-infected ryegrass is likely to directly affect the reproductive performance of breeding stock as well as reduce performance of finishing stock.

To date, only Eerens *et al.* (1994) have made observations on the effects of endophyte-infected ryegrass on lamb birthweight and subsequent growth rate to weaning. In that study there were no effects of endophyte on birthweight, but endophyte reduced lamb liveweight gain during the lactation period. The study was conducted in a cool, moist environment which may have reduced the adverse effects of endophyte on ewe and lamb performance.

There is a need to further investigate the effects of endophyte on lamb growth rate, prior to, and after puberty, as a likely source of reproductive loss. It is also necessary to examine these effects on animal performance in a range of environmental conditions to identify the existence of any endophyte by environment interactions

The objectives of this study were to examine the effects of grazing endophyte-infected ryegrass, prior to and during lactation, on ewe and lamb liveweight in a warm Northland environment.

2. Materials and methods

2.1. Animals and treatments

2.1.1. Animals

A Northland, commercial, mixed-age Coopworth ewe flock due to start lambing 1 September 1998 was pregnancy scanned in June 1998. From this flock, 100 twin-bearing ewes, which had conceived in the same oestrous cycle, were identified and separated out. The ewes were allocated, on a liveweight basis, to two even groups and grazed on endophyte-infected (E+) perennial ryegrass pasture until three weeks prior to the start of lambing.

The ewes and experimental pastures used in this trial were from the same commercial farm used for trials described in Chapter IV.

2.1.2. Treatment pastures

On 10 August (three weeks prior to the start of lambing) one group was introduced to an endophyte-free (E-) perennial ryegrass pasture where they were set-stocked at 5 ewes/ha until the end of November 1998 when the lambs were weaned. The remaining group was stocked at the same rate on E+ pasture until lamb weaning. The treatment pastures had the same average pasture cover when the animals were introduced.

2.2. Sampling

2.2.1. Animal measurements

Once a ewe had lambed, the lambs were weighed and ear-tagged. Any ewe or lamb deaths were recorded.

All ewes and lambs were weighed at docking (removal of lamb tails, and testicles in ram lambs, by application of a rubber ring) (7 October 1998) and again at weaning (23 November 1998).

Blood samples were taken at docking and at weaning by jugular venipuncture, from 20 ewes in each group to measure serum prolactin concentration.

2.2.2. Pasture measurements

Three random pasture samples (20 cm x 50 cm quadrats), cut to ground level, were taken from the E+ and E- pastures pre-treatment (10 August), at docking, and at weaning. The fresh weight of each sample was recorded and a 100 g (fresh-weight) subsample was taken, oven dried at 60°C, and reweighed to determine dry matter %. A second subsample was freeze-dried, and ground for nutritional analysis. A further subsample was dissected into ryegrass leaf blade and leaf sheath, freeze-dried, ground, and analysed for lolitrem B and ergovaline levels.

2.2.3. Prolactin analysis

Serum prolactin levels were measured at the Institute of Food and Human Health, Massey University, Palmerston North using a double-antibody radioimmunoassay (RIA) procedure based on the methods of Van Landegham & Van de Weil (1978). (see Chapter VI, section 2. for details).

2.2.4. Ergovaline and lolitrem B analyses

Freeze-dried and ground ryegrass samples were measured for ergovaline and lolitrem B levels by HPLC as described by Barker *et al.* (1993) at the Plant Biochemistry Laboratory, AgResearch Grasslands, Palmerston North (see Chapter III, section 2.5. for details).

2.2.5. Pasture nutritional analysis

Freeze-dried and ground pasture samples were analysed by NIR for protein, lipid, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble carbohydrate (CHO), organic matter digestibility (OMD), and metabolisable energy (ME) at the feed-TECH laboratory, AgResearch Grasslands, Palmerston North.

2.3. Statistical analyses

All statistical analyses were carried out using Graph-pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.
Ewe liveweight, serum prolactin, lamb birthweight, weaning weight and daily growth rate data were analysed by analysis of variance (ANOVA). The differences between pairs of groups in the ANOVA were determined by Tukey post test. Pasture nutritional parameters, ergovaline and lolitrem B concentrations were analysed by ANOVA.
Lamb survival rates were analysed by chi-square test.

3. Results

3.1. Ewe liveweight

The docking and weaning liveweights and numbers of ewes that raised either single, twin or no lambs (rearing status) are presented in Table 5.1.

There were no significant difference in mean (±SEM) pre-trial ewe liveweight between the E+ (54 ± 0.8 kg) and E- (55.3 ± 0.8 kg) groups.

At docking the E+ ewes were significantly ($P<0.05$) lighter than the E- ewes within the same rearing status. There were no significant differences between ewes of different rearing status within a treatment group.

At weaning the E+ ewes that had not reared lambs were significantly ($P<0.05$) lighter than the E- ewes than had not reared lambs. There were no significant differences in ewe liveweight between the E+ and E- groups for any other rearing status or between ewes of different rearing status within a treatment group. However, trends were for lighter E+ ewes than the E- ewes of the same rearing status.

Table 5.1. Mean (±SEM) liveweight (kg) and number (N) of lactating ewes of different rearing status grazing either endophyte-infected (E+) or endophyte-free (E-) ryegrass pasture at lamb docking and weaning.

	NUMBER OF LAMBS REARED					
	0		1		2	
Ewe Wt.	**E+**	**E-**	**E+**	**E-**	**E+**	**E-**
Docking	56.4±2.1^{ab}	64.9±2.8^{c}	57.3±1.4^{ab}	63.2±2.0^{c}	56.2±0.9^{a}	62.2±2.1^{bc}
Weaning	53.8±2.2^{a}	62.6±3.2^{bc}	55.5±1.5^{ac}	56.9±2.0^{ac}	55.3±0.9^{ac}	57.3±1.7^{ac}
N	2	6	8	17	38	24

Means with different superscript letters within a row differ significantly (P<0.05).

3.2. Lambing date and birthweight

There was no difference in mean lambing date between the treatment groups. Lambing started on 24 August and finished 15 September (mean lambing date = 4 September).
Lamb birthweight data are presented in Table 5.2. The data presented are the mean birthweights of all twin ram and ewe lambs born. Birthweights were not measured for 13 lambs in the E- group and 2 lambs in the E+ group as they were not identified at birth. There were 2 sets of triplet lambs and 2 single lambs in the E- group and 1 set of triplet lambs in the E+ group that were not included in the birth weight data due to their different birth rank.
E- ram lambs were significantly (P<0.05) heavier at birth than E+ ram lambs and E+ ewe lambs. The mean birthweight of ram lambs was greater than ewe lamb within a treatment group, but this was not significant. Birthweights for the ewe lambs trended towards higher birthweight in the E- group compared with the E+ group, but this also was not significant.

Table 5.2. Mean (±SEM) birthweight (kg) and the number (N) of ram and ewe lambs born to ewes grazing either endophyte-infected (E+) of endophyte-free (E-) pasture.

	RAM LAMBS		EWE LAMBS	
	E+	**E-**	**E+**	**E-**
BWT	3.89±0.12^{a}	4.22±0.11^{b}	3.79±0.10^{a}	3.95±0.13^{ab}
N	46	38	50	43

Means with different superscript letters differ significantly (P<0.05)

3.3. Lamb growth rate

The growth rates of ram and ewe twin lambs and lambs that were born as twins and lost a sibling, from birth to docking and from docking to weaning are presented for the E+ and E- groups in Table 5.3. Lambs that were born as twins and lost a sibling are subsequently referred to as singles. The growth rate of lambs that were born as triplets or singles are not included in the data.

Birth to docking

Between birth and docking there were no significant differences in the growth rate of lambs of the same sex and litter size between treatments. Growth rates were not significantly different between ram and ewe lambs from the same litter size, within each group. Single ewe lambs had significantly ($P<0.05$) higher growth rate than twin ewe lambs in the E+ group but not in the E- group. There were no significant differences in growth rate between single and twin ram lambs in either treatment group.

There was no significant difference in lamb growth rate between the E+ and E- groups when growth rates were pooled across sex and litter size within a group.

Docking to weaning

The growth rate of lambs in the E- group was significantly higher than the growth rate of lambs in the E+ group for all sexes and litter sizes with the exception of single ewe lambs. E+ single ewe lambs grew significantly ($P<0.05$) faster than E+ single ram lambs. There were no significant differences in growth rate between ewe and ram lambs for any other litter size in any treatment group. Single E+ ewe lambs grew significantly ($P<0.05$) faster than twin E+ ewe lambs, but the opposite was true for the ram lambs. There were no significant differences in growth rate between ewe or ram lambs of different litter size in the E- group.

The growth rate of E+ lambs was significantly ($P<0.001$) lower than E- lambs when growth rates were pooled across sex and litter size within a group.

Table 5.3. Mean (±SEM) liveweight gain (g/d) and the number (N) of twin and single, ram and ewe lambs in the E+ and E- groups between birth and docking (B-D) and docking to weaning (D-W) and pooled means for each group.

	TWIN LAMBS				SINGLE LAMBS				POOLED	
	RAM		EWE		RAM		EWE			
	E+	E-	E+	E-	E+	E-	E+	E-	E+	E-
ADG	221	207	213	211	219	267	314	232	223	218
(B-D)	$\pm 7^{ab}$	$\pm 13^{ab}$	$\pm 6^{ab}$	$\pm 19^{ab}$	$\pm 74^{ac}$	$\pm 23^{bc}$	$\pm 32^{c}$	$\pm 35^{ac}$	±5	±11
N	39	25	41	20	3	6	5	13	88	64
ADG	157	240	166	232	104	258	226	219	167	241
(D-W)	$\pm 7^{a}$	$\pm 12^{b}$	$\pm 8^{a}$	$\pm 11^{b}$	$\pm 35^{c}$	$\pm 23^{b}$	$\pm 14^{b}$	$\pm 16^{b}$	±5	±10*
N	36	24	38	20	3	6	5	11	82	61

Means (excluding pooled means) in a row with different superscript letters differ significantly ($P<0.05$).

*= Pooled mean differ significantly ($P<0.001$) between treatments.

3.4. Lamb weaning weights

Weaning liveweights of twin and single, ram and ewe lambs in the E+ and E- groups are presented in Table 5.4.

There were no significant differences in weaning liveweight between ram and ewe lambs within the same litter size and treatment group. Twin lambs in the E- group were significantly ($P<0.002$) heavier than E+ twin lambs at weaning. There were no significant differences between single lambs of different sex, litter size or treatment group. There were no significant differences between any of the groups of single lambs and any of the groups of twin lambs.

The weaning liveweight of E+ lambs was significantly ($P<0.001$) lower than E- lambs when weights were pooled across sex and litter size within a group.

Table 5.4. Mean (±SEM) liveweight (kg) at weaning (WW) and the number (N) of twin and single, ram and ewe lambs in the E+ and E- groups, and pooled means for each group.

	TWIN LAMBS				SINGLE LAMBS				POOLED	
	RAM		EWE		RAM		EWE			
	E+	E-	E+	E-	E+	E-	E+	E-	E+	E-
WW	19.7	22.6	18.9	22.0	17.2	24.4	24.6	21.0	19.6	21.9
	±0.5[a]	±0.8[b]	±0.5[a]	±0.9[b]	±4.9[ab]	±1.8[ab]	±1.6[ab]	±2.0[ab]	±0.4	±0.6*
N	36	24	38	20	3	6	5	11	82	61

Means (excluding pooled means) in a row with different superscript letters differ significantly ($P<0.05$).

*= Pooled means differ significantly ($P<0.001$) between treatments.

3.5. Lamb survival

Total numbers of lambs born, lambs lost between birth and docking, and between docking and weaning, and the total number of lambs lost are shown in Table 5.5.

More ($P<0.004$) lambs were found dead shortly after birth in the E- group than in the E+ group. The number of deaths of lambs that survived for more than one day after birth was not affected by endophyte status of the pasture between birth and docking, and docking and weaning. However, the greater number of unidentified lambs that were found dead in the E- group resulted in significantly ($P<0.006$) higher total lamb loses between birth and docking and significantly ($P<0.006$) lower numbers of live lambs at weaning in the E- group compared with the E+ group.

The causes of lamb deaths were not determined, however, the greater number of lambs that died from soon after birth to docking in the E- group was most likely due to a greater incidence of starvation/exposure. This was due to the E- pastures being in a wetter more exposed part of the farm.

Table 5.5. Number of lambs born, lamb deaths between birth and docking, and between docking and weaning, and the total lambs lost in the E+ and E- group.

	E+	E-
Total Lambs Born	101*	100*
Lambs found dead (unidentified)	2[a]	13[b]
Deaths, birth to docking (excluding unidentified)	9	16
Deaths docking to weaning	6	4
Total lambs lost	17[a]	33[b]
Total lambs weaned	84[a]*	67[b]*

Numbers in a row with different superscript letters differ significantly ($P<0.006$).

*Numbers include lambs born as triplets and singles.

3.6. Serum prolactin

There were no significant differences in serum prolactin at docking (256 ± 78 ng/ml and 280 ± 56 ng/ml for the E+ and E- ewes respectively) or weaning (206 ± 63 ng/ml and 198 ± 82 ng/ml for the E+ and E- groups respectively) between ewes grazing E+ ryegrass pasture and those grazing E- ryegrass pasture. There were no significant differences in serum prolactin between docking and weaning in either group of ewes.

Blood samples were taken from randomly selected ewes and made no allowance for single or twin rearing ewes or the stage of lactation.

3.7. Pasture dry matter cover

There were no significant differences in average pasture dry matter cover between the E+ and E- pastures at docking (1891 ± 209 kgDM/ha and 1489 ± 412 kgDM/ha for the E+ and E-

pastures respectively). There was a significantly ($P<0.05$) greater pasture cover on the E+ pasture at weaning than on the E- pasture (2112 ± 264 kgDM/ha and 1189 ± 212 kgDM/ha for the E+ and E- pastures respectively).

3.8. Herbage ergovaline and lolitrem B concentration

Mean (±SEM) ergovaline concentration in the E+ pasture was 0.20 ± 0 ppm and 0.80 ± 0.20 ppm for the ryegrass leaf blade and leaf sheath respectively. There was no ergovaline detected in any of the E- pasture samples. Mean (±SEM) lolitrem B concentration in the E+ pasture was 0.75 ± 0.08 ppm and 3.32 ± 0.08 ppm for the ryegrass leaf blade and leaf sheath respectively. No lolitrem B was detected in any E- pasture sample.

Ergovaline and lolitrem B concentrations in the E+ ryegrass were significantly ($P<0.001$) higher in the leaf sheath than the leaf blade.

3.9. Pasture nutritional analysis

Mean nutritional parameters in the E+ and E- pastures during the trial period are presented in Table 5.6.

As there were no differences in treatment results between sampling dates, results are expressed as treatment means.

There was no difference between the E+ and E- pasture in any of the nutritional parameters measured during the trial period. There was no difference between sampling dates in any nutrition parameter measured in the E+ and E- pasture.

Table 5.6. Mean (±SEM) protein (PROT), lipid, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble carbohydrate (CHO), organic matter digestibility (OMD) and metabolisable energy (ME) in Northland E+ and E- ryegrass pasture.

	E+	E-
PROT	13.5 ± 2.0	9.7 ± 0.6
LIPID	3.2 + 0.5	2.9 + 0.2
ASH	11.7 ± 1.5	10.2 ± 1.1
ADF	28.8 ± 2.2	27.2 ± 2.6
NDF	48.2 ± 1.6	50.3 ± 0.1
CHO	7.8 ± 1.1	8.0 ± 2.1
OMD	77.0 ± 1.1	75.6 ± 0.6
ME	11.5 ± 0.2	11.3 ± 0.1

4. Discussion and conclusions

4.1. Ewe liveweight

Between the start of the treatment period (10 August) and docking (7 October) the E- ewes gained an average of 6.3kg per ewe whereas the E+ ewes' liveweight did not change significantly. As a consequence the E- ewes were significantly heavier than the E+ ewes at docking across all rearing-ranks.

Pasture measurements indicated that the amount and nutritional quality of the feed available during this period were not different between the treatments and, therefore, were unlikely to have caused differences in ewe liveweight. The most likely cause for the differences in ewe liveweight at docking is higher feed intakes in the E- ewes between the start of treatments and docking. Feed intakes were not measured during this trial so this could not be verified as the cause. However, differences in feed intake between lactating ewes grazing either E+ or E- pasture have subsequently been observed in a later trial (see Chapter VIII).

Greater liveweight gains in the lactating E- ewes may also have been aided by the greater proportion of single-rearing (lost one of a set of twins) and dry (lost all lambs) ewes in this group compared with the E+ group. Although rearing rank did not appear to significantly

affect docking liveweight within each group it is possible that the reduction in nutritional requirements of the ewes feeding fewer lambs in the E- group will have allowed a greater amount of feed for liveweight gain. Additionally, lamb deaths resulted in 18 fewer lambs in the E- group than in the E+ group and, in particular, fewer sets of twins. Lower numbers of twins in the E- group will have reduced the amount of pasture required by the lamb population in the E- group. Twin lambs rely on pasture as a greater portion of their diet, compared with single lambs, with individual twins consuming only about 60% of the milk consumed by single lambs and, therefore, substituting with more pasture (Geenty & Dyson, 1986). Pasture can make up a significant (30%) part in the diet in twin lambs from as early as three weeks of age (Geenty *et al.*, 1985). It is not clear what impact the reduction in lamb numbers had on the pasture supply for the E- group, however, it is likely that the losses resulted in a significant increase in the pasture availability for the remaining ewes and lambs. This is in addition to reducing the lactation requirements of many of the ewes.

Between docking and weaning the E- ewes lost an average of 5kg per ewe whereas the E+ ewes maintained their liveweight. This resulted in no significant differences in liveweight at weaning between E+ and E- ewes that reared lambs. The dry ewes in the E- group had not lost as much weight between docking and weaning as ewes that had reared a lamb in the E- group and were still significantly heavier than the dry ewes in the E+ group.

The weight loss in the E- ewes rearing lambs appeared to coincide with better lamb growth rates than ewes in the E+ group. It is possible that the E- ewes were producing more milk during this period than the E+ ewes and that this was expressed as a reduction in ewe liveweight and higher lamb growth rates. As dry matter covers decreased between docking and weaning on the E- pasture, feed intakes would have fallen and this would have increased the reliance of the E- ewes on body reserves for milk production. Additionally, it is possible that the E- lambs were consuming more pasture individually than the E+ lambs. Feed intakes in suckling lambs have been shown to be higher on E- pasture than on E+ pasture (see Chapter VIII). Greater feed intake in the E- lambs may have put a greater pressure on the feed supply that may in turn have limited the ewe intakes further and contributed to the liveweight reductions.

4.2. Lamb birthweight, growth rate, and survival

Mean birthweights were higher in the E- lambs than in the E+ lambs, although this was only significant for the ram lambs.

Better birthweights in the E- lambs may have been due to better ewe liveweight and liveweight gain during the last three weeks of pregnancy. It has previously been shown that greater ewe liveweight gain during the last six weeks of pregnancy is associated with significant increases in the birthweight of both single and twin lambs (Scales *et al.,* 1986). Other possible reasons for the difference in birthweight between the E+ and E- ram lambs are not obvious. Eerens *et al.* (1994) found no differences in lamb birth weight between ewes grazing E+ and E- pastures, however, there were also no significant differences in prelamb ewe liveweight gains in this trial and lamb birthweights were not reported for each sex.

Mean birthweights were higher in the ram lambs than in the ewe lambs within each group, although this was not significant. The trend for higher birthweight in the ram lambs in the present study agrees with previous studies, which showed that sex of the lamb is a significant factor in the determination of birthweight in sheep (Bendicho de Combellas *et al.,* 1979; Everts *et al.,* 1985; Lopez *et al.,* 1990).

There were no significant differences in lamb liveweight gain between the E+ and E-groups from birth to docking. However, E- twin and single lambs grew significantly faster than all E+ lambs between docking and weaning. These results are consistent with other reports that found lower growth rates of suckling-lambs on E+ pasture compared with E- pasture (Eerens *et al.,* 1994; Watson *et al.,* 1999). There were no significant differences in growth rate between single and twin lambs within a group, with the exception of ewe lambs in the E+ group, which is in contrast to other reports (Watson *et al.,* 1999). There were small numbers of single lambs raised relative to twin lambs which limits the comparison, additionally, the single lambs were born as twin lambs and the impact of this on subsequent growth rate can not be determined.

Greater growth rate in the E- lambs may be associated with increased milk production in the E- ewes due to better body condition and feed intake. Lower dry matter cover on the E-

pasture than on the E+ pasture at weaning could indicate greater intakes in the E- group, but pasture growth rates were not measured.

If differences in milk production between E+ and E- ewes did exist, they did not appear to be expressed in lamb growth rate between birth and docking. This may have been due to the wetter and colder conditions on the E- area that could have reduced lamb vigour and increased the energy requirements of the lamb for generating heat, at the expense of growth.

Differences in milk production between twin-rearing ewes grazing either E+ or E- ryegrass have been observed and this was associated with differences in ewe condition and an apparent difference in feed intake (see Chapter III).

It is also possible that the E- lambs were consuming more pasture than the E+ lambs. Feed intakes of suckling-lambs during late lactation have subsequently been found to be higher on E- pasture than on E+ pasture (see Chapter VIII). Therefore the higher growth rates in the E- lambs may also be a reflection of the higher herbage intake in these animals, which could have become more evident as weaning approached and the lambs became more reliant on pasture for nutrition.

The overall better liveweight gain in the E- lambs has important implications for both finishing and breeding stock policies. Heavier lambs at weaning mean a greater number of lambs could be slaughtered earlier at a higher weight, and replacement breeding ewes would achieve puberty earlier and have a better lifetime production.

It is likely that lamb growth rates and consequently weaning weights would have been considerably better in the E- lambs had the weather conditions been better during the early stages of lactation.

Lamb survival was significantly lower in the E- group than in the E+ group and, as a result, more lambs were weaned from the E+ group. This higher incidence of lamb mortality in the E- group occurred between birth and docking when there was a period of bad weather. The area where the E- pasture was sown was more exposed to wind and provided little shelter from rain compared with the E+ pastures. This resulted in a greater number of deaths due to hypothermia in the E- group. Thirteen lambs in the E- group died soon after birth during

periods of bad weather compared with only 2 lambs in the E+ group. A greater number of lambs died in the E- group due to misadventure, which was generally falling into drains that contained water from rain run-off.

Between docking and weaning the weather conditions were dry and mild and there were very few lamb deaths in either group during this period.

It can be concluded that differences in lamb mortality rate between the treatment groups in this trial were a result of differences in the exposure to environmental conditions rather than any treatment effects.

Greater growth rate in lambs reared on E- pasture recorded in this trial is consistent with findings in other trials.

4.3. Conclusions

Results in this trial generally support other reports that ewe liveweight and lamb growth rates are better on E- pasture than on E+ pasture. Limitations in the trial design with respect to pasture measurement and the lack of milk production data obscures possible reasons for the observed differences.

There were also difficulties in the interpretation of some of the results as environmental conditions varied between the pasture treatments that resulted in higher lamb mortality and may have reduced lamb growth rate in the E- group.

CHAPTER VI

Effects of long-term grazing of endophyte-infected perennial ryegrass on serum lutenizing hormone, progesterone and prolactin levels in cyclic ewes

1. Introduction

One of the more serious consequences of grazing endophyte-infected tall fescue pasture is reduced reproductive performance. This may also be the case in animals grazing E+ perennial ryegrass pastures.

The mechanisms by which endophyte toxins affect the reproductive performance of grazing animals are still not well understood.

Endophyte toxin production is highest during the late summer and autumn. This increase in toxin production coincides with mating in many New Zealand ewe flocks. It is possible that exposure to endophyte toxins at this time may disrupt some of the endocrinological processes controlling the oestrous cycle. The depression of serum prolactin in several species of domestic animals grazing E+ pasture is well documented (Redmond *et al.*, 1994; Bond & Bolt, 1986: Bolt *et al.*, 1982). It has also been reported that endophyte toxins affect the production of luteinizing hormone (Browning *et al.*, 1997) and progesterone in cattle (Mahmood *et al.*, 1994). These hormones have important roles in the regulation of the oestrous cycle but with the exception of prolactin, there have been no reports on the effects of endophyte toxins on these hormones in sheep.

Ewes grazing pasture containing ergovaline during mating have reduced ovulation rate and number of lambs carried at ninety days gestation (Kramer *et al.*, 1999). This reduction in ovulation rate may be due to disruptions in the endocrine regulation of the hypothalamic-pituitary-ovarian axis.

This study was conducted to determine whether there are differences in the profiles of prolactin, luteinizing hormone, and progesterone in cyclic ewes grazing either E+ or E- ryegrass pasture at mating.

2. Materials and methods

2.1. Animals and treatments

Twenty, four-year-old Romney ewes of the same average liveweight were selected from each of two groups of ewes that had been grazing either endophyte-infected (E+) or endophyte-free (E-) perennial ryegrass pasture for the previous three years. Once the ewes had been weighed and identified with ear-tags they continued to be grazed on either E+ or E- perennial ryegrass pastures.

On 18 February 1999, each ewe was treated with an intra-vaginal progesterone (0.3 g) release device (EAZI-BREED CIDR-G, InterAg ©) for fourteen days to synchronise oestrous. At CIDR withdrawal (5 March 1999) two vasectomised teaser rams equipped with harness and crayon were introduced to each group to determine the onset of oestrus. After all ewes had exhibited oestrous the teaser rams were removed and replaced with entire rams also equipped with harness and crayon, to detect returns to oestrus.

2.2. Sampling

2.2.1. Blood

After CIDR-G withdrawal, each ewe was blood sampled by jugular venipuncture using a hypodermic needle and 10 ml, non-heparinised, evacuated blood collection tubes (Vacutainer ®). Daily blood samples were collected from all ewes between 0900 am and 1000 am for the next twenty-one days.

Once collected, blood samples were chilled on ice, spun at 3000 rpm for 30 minutes and the serum was removed and stored at –20°C in 1ml aliquots (n=4 per blood sample) until required for LH, progesterone and prolactin analysis.

2.2.2. Rectal temperature

Rectal temperatures were measured by digital thermocouple thermometer every second day throughout the duration of the trial. Daily maximum and minimum ambient temperature and the ambient temperature at sampling were also recorded.

2.2.3. Herbage

Ryegrass tillers (n=50) were collected from the E+ and E- pastures every week during the trial period. Each sample was dissected into ryegrass leaf blade and leaf sheath, freeze-dried and ground before HPLC analysis for lolitrem B and ergovaline at the Plant Biochemistry Laboratory, AgResearch Grasslands, Palmerston North, New Zealand.

2.3. Hormone analyses

The blood samples for both treatment groups were divided into three batches according to stage of the oestrous cycle at sampling (Batch 1 = days 1-7, B2 = 8-14, and B3 = 15-21) and analysed in separate assay runs. Blood samples within a batch were placed at random in each assay run.

2.3.1. Progesterone

Serum progesterone levels were determined using a solid-phase ^{125}I radio-immunoassay kit (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA) in accordance with the manufacturer's instructions, at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand.

Four uncoated polypropylene 5 ml tubes were labelled T (total counts) and NSB (non-specific binding) in duplicate. Fourteen polypropylene tubes coated with rabbit antibodies to progesterone (Ab-Coated) were labelled A (maximum binding) and B to G in duplicate. Additional Ab-Coated tubes were labelled in duplicate for the controls and samples.
100 µl of the zero calibrator A was pipetted into the NSB and A tubes, and 100 µl of the calibrators B to G was pipetted into the correspondingly labelled tubes (Table 6.1.).

Table 6.1. Progesterone standards

Calibrators	Progesterone (ng/ml)
A	0
B	0.1
C	0.5
D	2
E	10
F	20
G	40

100 µl of each of the control and unknown samples were pipetted into the prepared Ab-Coated tubes.

1.0 ml of ^{125}I progesterone (iodinated progesterone) was added to all tubes followed by vortex mixing.

The tubes were incubated at room temperature (15 – 28 °C) for 3 hours and then all tubes (except the T tubes) were decanted and struck sharply on absorbent paper using a foam decanting rack to remove any residual droplets.
The tubes were then counted for 1 minute in a gamma counter.

The inter-assay co-efficient of variation (%CV) (calculated from progesterone standards run in each assay) was 5.36%. The intra-assay %CV (calculated from average %CV of the sample duplicates) was 3.92%. Samples that had concentrations below the lowest standard (0.1 ng/ml) had an intra-assay %CV of 24.1% but represented a small portion of the total samples analysed. Samples with %CV of >20% were repeated.

2.3.2. Prolactin

Serum prolactin levels were determined using a double-antibody ^{125}I radioimmunoassy procedure based on the methods of Van Landegham & Van de Weil (1978) at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand.

The following reagents were added to each tube: 50 µl containing 0.1 ng [^{125}I] oPRL (10000 cpm); 50 µl of antiserum (1:50000) (except tubes for non-specific binding); 50 µl of standard (0.1 to 5 ng oPRL (NIADKK-oPRL-I-2, (NIH, Bethesda, MD, USA)) or an appropriate volume of plasma sample (2 to 100 µl) and 1% BSA-tris buffer to bring the total volume to 500 µl.

The tubes were incubated at 4°C for 4 days. After this 1 ml of the second antibody solution (5.5 ml double antibody/solid phase (DASP) diluted to 50 ml with 0.1% bovine serum albumin (BSA)-tris buffer) was added and tubes were incubated at 4°C for 1 night.

The tubes were centrifuged at 1700 g for 5 mins. The supernates were aspirated by vacuum and precipitates were washed twice with 2 ml of tris-buffer. The precipitates containing bound hormone were counted for 5 mins on a gamma counter.

The inter-assay %CV was 12.9% and was. The intra-assay %CV was 9.5%. Samples with %CV of >20% were repeated (CVs were provided by Phil Pearce, IFNHH, Massey University).

2.3.3. Luteinizing hormone (LH)

Serum ovine LH levels were determined using a ^{125}I radioimmunoassy at the Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University, Palmerston North, New Zealand. The assay used was a four-day, 50:20:20:20 system.

DAY1

Forty-eight, 5 ml plastic tubes were labelled A to P in triplicate.

50 µl of diluent I (Phosphate buffer with BSA (5 g/ml)) was pipetted into tubes B (non-specific binding (NSB)) and C (Zero).

50 µl of ovine LH standard (McNeilly, @ 10 ng/mL) was pipetted into tubes D and E which were serially diluted with diluent I through to tube M (Table 6.2.).

Table 6.2. Luteinizing hormone standards

TUBE	LH STANDARD (ng/ml)
D	10
E	5
F	2.5
G	1.25
H	0.6250
I	0.3125
J	0.1560
K	0.0780
L	0.0390
M	0.0195

50 µl of ovine hormone-free serum (oHFS), high ovine LH (oLH) control and low oLH control was pipetted into tubes N, O and P respectively.

Sample tubes were numbered in duplicate and 50µl of each serum sample was pipetted into their corresponding tube.

20 µl of normal rabbit serum (NRS) diluted 1:400 in diluent I was added to the NSBs (tube B).

20 µl of anti-oLH antiserum R29 diluted 1:150,000 in the 1:400 NRS was added to the remaining tubes.

All tubes were vortex mixed and left overnight at 4°C.

DAY 2

20 µl of diluted ^{125}I-oLH label (5000 counts/minute/20 µl) was added to all tubes. The totals (tube A) were capped and all tubes were vortex mixed and left overnight at 4°C.

DAY 3

20 µl of donkey anti-rabbit serum (DARS) diluted 1:60 in diluent I was added to all tubes (except totals), vortex mixed and left overnight at 4°C.

DAY 4

200 µl of diluent IV (diluent I with egg album (5 g/ml)) was added to all tubes (except totals). The tubes were spun in a centrifuge at 3750 rpm for 1 hour at 4°C. After spinning, the supernatant in each tube was aspirated and the remaining pellet was counted on a gamma-counter for 2 minutes/tube.

The inter-assay co-efficient of variation (%CV) (calculated from high, medium and low control samples run in each assay) was 22.49%. The intra-assay %CV (calculated from average %CV of the sample duplicates) was 6.49%. Samples with %CV of >20% were repeated.

2.4. Statistical analyses

All statistical analyses were carried out using Graph-pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.

Progesterone, prolactin and LH data were analysed by fitting a generalised linear model to determine sources of variation due to treatment (endophyte), time (day of oestrous cycle) and treatment X time interactions.

3. Results

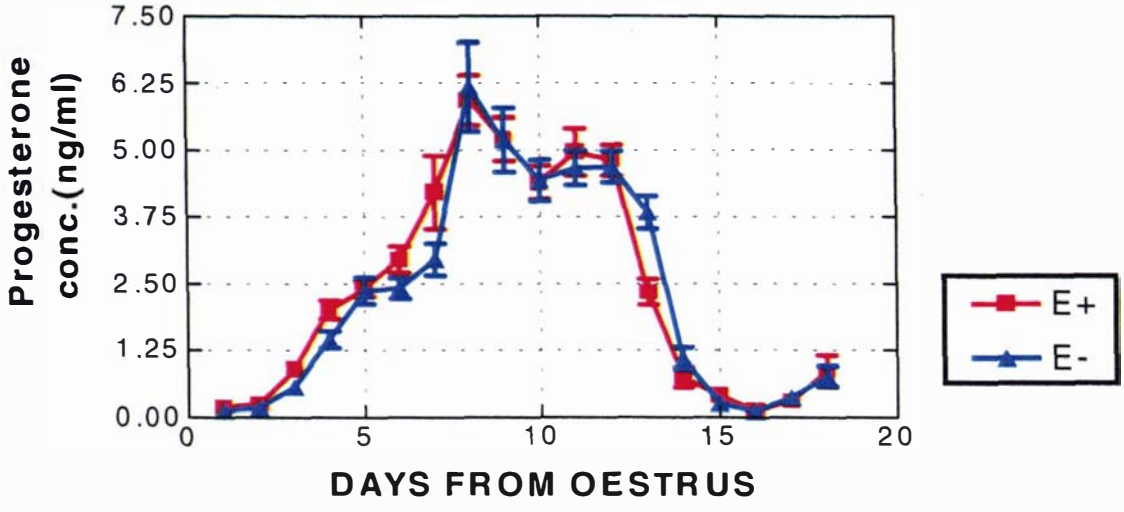
3.1. Serum progesterone

Daily serum progesterone in the E+ and E- ewes during the oestrous cycle are shown in Figure 6.1.

Serum progesterone levels were significantly higher ($P<0.05$) in the E+ ewes between days 1 and 7 after oestrus. Peak progesterone levels were reached on day 8 post-oestrus in both the E+ and E- ewes and there was no significant difference in peak serum progesterone levels. Serum progesterone levels did not change significantly between days 9 and 12, during which time there was no difference between the E+ and E- ewes. Progesterone levels decreased between days 13 and 16, during which period levels were significantly ($P<0.05$) lower in the E+ ewes.

There was a significant (P<0.001) effect of time (day from oestrus) on serum progesterone levels and a significant (P<0.1) time X treatment interaction.

Figure 6.1. Mean (±SEM) serum progesterone levels in cyclic ewes grazing either E+ or E- perennial ryegrass pasture.

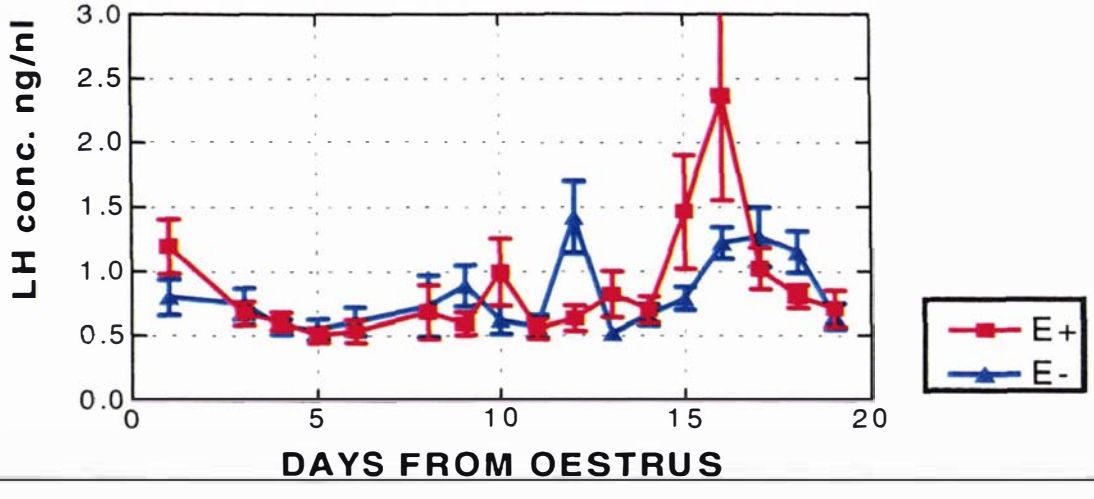


3.2. Serum luteinizing hormone (LH)

Daily serum LH in the E+ and E- ewes during the oestrous cycle is shown in Figure 6.2.

There was no significant difference in serum LH levels between the E+ and the E- ewes at any time during the oestrous cycle. There was a significant (P<0.001) effect of time (day from oestrus) on serum LH and no significant time X treatment interaction.

Figure 6.2. Mean (±SEM) serum LH levels in cyclic ewes grazing either E+ or E- perennial ryegrass pasture.

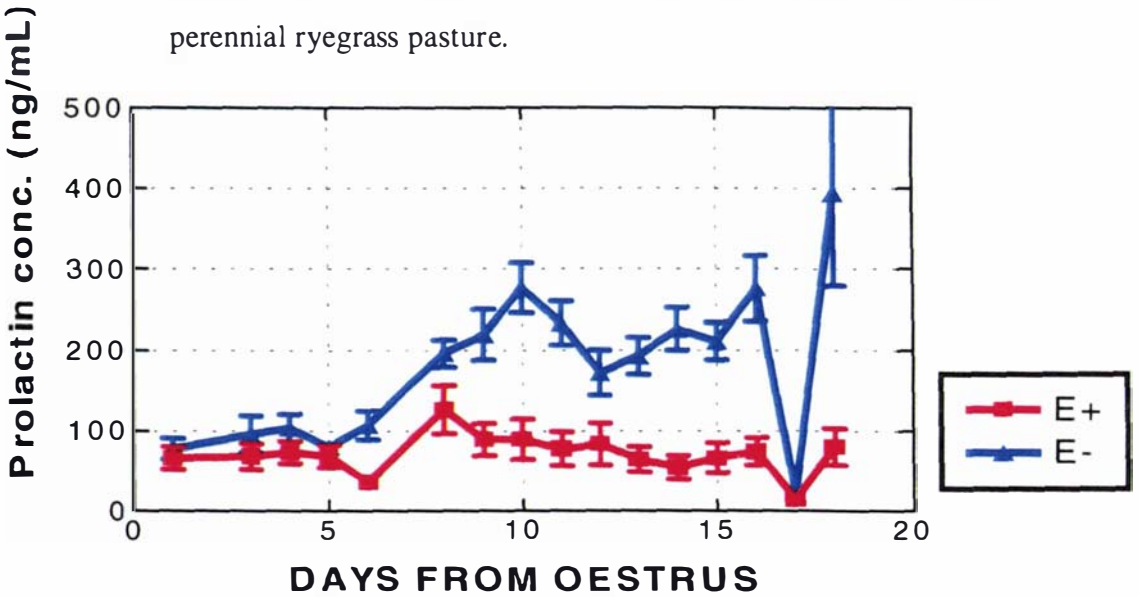


3.3. Serum prolactin

Daily serum prolactin in the E+ and E- ewes during the oestrous cycle are shown in Figure 6.3.

Serum prolactin levels were significantly (P<0.0001) lower in the E+ ewes than the E- ewes during the oestrous cycle. Day of oestrous had a significant (P<0.0001) effect on serum prolactin levels and there was a significant (P<0.0001) treatment (endophyte) X time (day from oestrus) interaction (see Figure 6.3)

Figure 6.3. Mean (±SEM) serum prolactin levels in cyclic ewes grazing either E+ or E- perennial ryegrass pasture.



3.4. Ambient and ewe body temperature

The mean (±SEM) maximum daily ambient temperature during the trial period was 25.7 (±1.1)°C (range: 22.7°C – 29.8°C) and the mean minimum ambient temperature was 11.3 (±0.7)°C (range: 8.9°C – 13.2°C). The mean ambient temperature during blood sampling was 18.6 (+0.8)°C (range: 15.4°C – 22.0°C).

There were no significant differences in the rectal temperature of ewes between treatments or between days of sampling. Mean (±SEM) rectal temperatures of the E+ and E- ewes during the trial period were 38.7 ± 0.1°C and 38.8 ± 0.1°C respectively.

3.5. Pasture ergovaline and lolitrem B levels

Mean (±SEM) ergovaline concentrations in the E+ pasture during the blood sampling period were 0.6 ± 0.1 ppm and 1.4 ± 0.2 ppm for the ryegrass leaf blade and leaf sheath respectively. No ergovaline was detected in the E- pasture.

Mean (±SEM) lolitrem B concentrations in the E+ pasture during the blood sampling period were 1.7 ± 0.1 ppm and 4.3 ± 0.2 ppm for the ryegrass leaf blade and leaf sheath respectively. No lolitrem B was detected in the E- pasture.

4. Discussion and conclusions

4.1. Serum progesterone

Although peak serum progesterone levels and the overall pattern of progesterone secretion during the oestrous cycle was similar between the two groups, there were small significant differences in serum progesterone levels between the E+ and E- ewes on days 0 to 8, and 13 to 16 after oestrus. These differences may indicate differences in luteal function between the two groups.

To date, there have been no reports on the effects of grazing endophyte-infected pasture on serum progesterone in sheep. However, there have been some conflicting reports on serum progesterone levels in cattle grazing E+ tall fescue pastures. Mahmood *et al.* (1994) found differences in progesterone profiles in cyclic heifers grazing E+ and E- pasture and suggested that this was due to luteal dysfunction in the animals grazing the E+ pasture. Conversely, Fanning *et al.* (1992) found that grazing E+ pasture had no effects on serum progesterone levels in beef heifers.

To date, investigations into the effects of various endophyte toxins on hormones have focused on the pituitary-related endocrine systems and there is a lack of research into other important reproductive hormones such as progesterone.

Buys *et al.* (1990) found that injecting bromocriptine, which has similar physiological activity to ergovaline, from five days before until five days after mating did not affect serum

progesterone in ewes. However, this was a relatively short exposure to the toxin and it is possible that longer exposure prior to the luteal phase may be necessary to affect progesterone secretion. In an E+ pasture, toxins other than ergovaline and lolitrem B exist, which include other ergopeptine alkaloids and lolitrems, lysergic acid amides, and probably toxins that are yet to be identified. Very little is known about the effects of these on hormone secretion. Serum progesterone levels in animals grazing E+ pasture may also be affected by heat stress. Hill & Alliston (1981) found that cyclic ewes suffering heat stress had significantly lower serum progesterone levels. However, there were no significant differences in body temperature in this trial, which makes the difference in progesterone secretion unlikely to be caused by heat stress in the E+ animals.

Effects of endophyte toxins on serum progesterone levels in this trial may not have been severe enough to disrupt the reproductive system of the ewe. However, progesterone is an important hormone in regulating the oestrous cycle and in the establishment and maintenance of pregnancy. Therefore, any effects endophyte toxins may have on secretion of this hormone should be examined closely and not be discounted as a possible mechanism by which poor reproductive performance is caused in animals grazing E+ pasture.

4.2. Serum luteinizing hormone

As for serum progesterone, there are no reports on the effects of grazing endophyte-infected pasture or specific endophyte toxins on serum LH for sheep and few reports for cattle, and these have generally found no differences in LH levels between cows grazing E+ or E- pasture (Christopher *et al.*, 1990; Mizinga *et al.*, 1992).

Browning *et al.* (1998) found that ergot alkaloids similar to those found in endophyte-infected pasture, given in a single intravenous dose, lowered serum LH levels in cows during the luteal phase of the oestrous cycle. These depressions in serum LH occurred three to four hours after dosing depending on the ergot alkaloid administered. This showed that alkaloids such as those found in E+ pasture are capable of affecting serum LH secretion in cattle but these compounds were administered intravenously in a pure form at a relatively high concentration and may have very different effects from alkaloids naturally ingested by animals grazing E+ pasture.

In contrast to the Browning's findings was a study by Mi- zinga *et al.* (1992) that found no effect of feeding endophyte-infected fescue seed on LH secretion during the luteal or follicular phase in heifers. The contrasting nature of these two studies may indicate the difference in effects of endophyte toxins when artificially and naturally administered.

There is a need for more work to be done examining more closely the effects of endophyte toxins dosed, and as a natural constituent of pasture, on hormones such as LH and progesterone.

The examination of LH in sheep grazing E+ or E- pastures in this trial was a preliminary investigation to identify any major differences. The trial showed no differences but the once daily blood sampling regime was not sufficient to examine detailed differences in LH secretion profiles such as basal levels, pulse frequency, and amplitude during the oestrous cycle. As the secretion of LH is pulsatile and can have large diurnal variation, subsequent studies would require serial blood sampling during a 24-hour period to identify differences in secretion between groups of animals.

4.3. Serum prolactin

The suppression of serum prolactin is one of the most commonly reported effects in animals grazing E+ pastures. However, this is the first trial that examines serum prolactin profiles in ewes grazing E+ pasture during the oestrous cycle.

Serum prolactin is very sensitive to low levels of ergovaline and is used as an indicator of ergopeptine alkaloid intoxication in animals grazing E+ pasture (Fletcher & Easton, 1997). However, the depression of serum prolactin in sheep grazing E+ pasture is generally not detected unless ambient temperature is above 22°C (Fletcher *et al.*, 1996).

In cyclic ewes, detection of differences in serum prolactin levels between ewes grazing E+ and E- pasture is made more difficult by variation in prolactin secretion due to ewes being at different stages of the oestrous cycle. Average prolactin levels in this trial ranged from 70 ng/mL to more than 400 ng/ml in the E- ewes, and from 33 ng/ml to 150 ng/ml in the E+ ewes, depending on the stage of oestrous. There was a sharp fall in serum prolactin levels in both groups on day 17 that cannot be explained. Prolactin levels were lowest (70-100 ng/ml)

in the E- group during the 5 days after the 1st oestrus of the measurement period, and then began to rise (with the exception of the fall on day 17) steadily to peak levels (400 ng/ml) at the next oestrus. Serum prolactin levels were often two to three times lower in the E+ ewes than in the E- ewes for any given day of the oestrous cycle. Prolactin levels in the E+ ewes did not exceed 110 ng/ml and did not show the normal increase towards ovulation. This indicates a strong suppression when prolactin levels would normally be increasing steadily as ovulation approached. This type of suppression is also demonstrated in animals grazing E+ pasture that are subjected to high ambient temperature: prolactin in sheep grazing E-pasture increases rapidly, while sheep grazing E+ pasture fail to respond (Fletcher *et al.,* 1997).

Daily ambient temperatures during the trial were frequently greater than 22°C, (mean 25.7°C) which may have in part been responsible for the differences in serum prolactin. However, average night temperatures were generally low (11.3°C) during the trial period and there was no significant difference in body temperature of the ewes at blood sampling, which was conducted between 0900am and 1000am when ambient temperatures had not usually risen above 20°C (mean 18.3°C).

Although there were relatively large differences in serum prolactin levels between the E+ and E- ewes, the suppression of prolactin was not as severe as in cyclic ewes grazing E+ tall fescue pasture with ergovaline concentration of 3ppm (Kramer *et al.,* 1999). The prolactin response to ergovaline is characterised by a low threshold, with maximum depression occurring at or below 0.5 ppm ergovaline in an E+ ryegrass dominant pasture (includes leaf blade and leaf sheath components) (Fletcher & Easton, 1997).

The ergovaline concentration in the E+ pastures in this trial were above this level (see section 3.5.) however, serum prolactin levels in the E+ ewes were not suppressed to the extent observed in the trial by Kramer *et al.* This may have been due to differences in ambient temperature between the trials or other unidentified factors unique to E+ tall fescue.

There is evidence that prolactin has an important role in regulation of the oestrous cycle and ovulation. Ewes that had their prolactin secretion suppressed by daily injections of bromocriptine had a reduction in ovulation rate at induced oestrus (Rodway *et al.,* 1983). Polkowska *et al.* (1976) concluded that the pre-ovulatory rise in prolactin in the ewe had important roles at this stage of the oestrous cycle. This conclusion is supported by Rowday *et*

al. (1983), who found a significant correlation between pre-ovulatory peak values of prolactin and ovulation rate in ewes treated with bromocriptine.

Ergovaline suppresses prolactin secretion in a similar manner to bromocriptine Therefore it is possible that the suppression of prolactin in ewes grazing E+ pasture during the oestrous cycle, and in particular at the pre-ovulatory prolactin surge, may cause reductions in ovulation rate. Kramer *et al.* (1999) found significant reductions in ovulation rate in ewes grazing E+ tall fescue were associated with an almost total suppression of serum prolactin.

Ovulation rates were not determined in this trial so it could not be determined whether fertility was affected by the suppression of prolactin. Future trials examining the effects of endophyte toxins on reproductive hormone profiles during the oestrous cycle should include the measurement of reproductive parameters such as ovulation rate and returns to oestrous.

4.4.Conclusions

Grazing endophyte-infected ryegrass pasture had a significant suppressive effect on basal and pre-ovulatory serum prolactin levels in cyclic ewes. There also appeared to be small effects on serum progesterone levels suggesting some difference in luteal function between the E+ and E- ewes.

There were no significant differences in serum LH, although the blood sampling regime may not have been sufficient to detect differences in LH.

This trial showed that grazing E+ pasture affects hormones associated with the oestrous cycle and it is possible that the disruption of these hormones may be a major contributing factor in the reduction of reproductive performance.

CHAPTER VII

Effects of ergovaline and ambient temperature on selected hormones associated with mammary development in the ewe, and on lamb performance.

1. Introduction

Reduced milk production has been reported in cattle and sheep grazing E+ tall fescue pastures (Strahan *et al.*, 1987; Schmidt *et al.*, 1986; Stilham *et al.*, 1982). There have also been conflicting reports on milk production in cattle grazing E+ perennial ryegrass pastures. Some of these reports have shown significant reductions in the milk production of cows grazing E+ ryegrass pasture compared with E- ryegrass pasture (Keogh *et al.*, 1999; Clark *et al.*, 1996; Valentine *et al.*, 1993) while others have reported no significant differences (Thom *et al.*, 1999; Clark *et al.*, 1999; Thom *et al.*, 1994). Some of the variation between these may have been due to differences in duration of the treatments and endophyte levels in the treatment pastures.

Possible causes for reduced milk production in animals grazing E+ pasture could be reduced feed intake and/or disruption of the endocrine regulation of mammogenesis and lactogenesis.

Keogh *et al.* (1999) suggested that a hypothesised 20 - 25% reduction in feed intake may be a major factor in reduced milk production of cows grazing E+ perennial ryegrass pastures. Although reduced feed intake is likely to be a major determinant of milk production in animals grazing E+ pasture, Valentine *et al.* (1993) reported lower milk production in cows grazing E+ ryegrass which did not appear to be associated with differences in dry matter intake. Therefore, the effects of endophyte toxins on hormones associated with mammary development and lactation should not be ignored as factors contributing to reduced milk production in animals grazing E+ pasture.

Prolactin, progesterone, cortisol, oestradiol and insulin are important hormones in mammary development during late pregnancy in the ewe (Hart, 1976: Hart & Morant, 1980; Mellor *et al.*, 1987)

The suppressive effects of the ergopeptine alkaloids produced in E+ ryegrass pasture on serum prolactin in sheep are well documented (Fletcher & Easton, 1997). However, there have been no reports on the effects of endophyte toxins on the other major hormones responsible for lactation in the ewe. There is evidence that serum insulin levels are affected by the synthetic ergot alkaloid, bromocriptine, in rats (Flint *et al.,* 1981), humans (Zampa *et al.,* 1981) and *prepartum* ewes (Peterson, 1992). Therefore, it is possible that naturally occurring ergot alkaloids such as ergovaline may also affect insulin levels in grazing animals. It is possible that sufficient exposure to endophyte toxins prior to parturition will adversely affect mammary development and thus subsequent milk production.

It is well known that high ambient temperature interacts with endophyte toxins to either precipitate or exacerbate the toxic effects (Oliver, 1997). This appears particularly evident in the suppression of prolactin by the ergopeptine alkaloids. Fletcher *et al.* (1996) found that ambient temperatures above 22°C were necessary before significant differences in serum prolactin between sheep grazing E+ and E- ryegrass could be detected. Normally an increase in ambient temperature is associated with an increase in serum prolactin in sheep, which raises the possibility that temperature is a major factor in the control of plasma prolactin levels in sheep (Hill & Alliston, 1981). However, it is likely that the ergopeptine alkaloids produced by the endophyte prevent this response. It is, therefore, possible that increased ambient temperature will exacerbate any detrimental effects endophyte toxins may have on the hormones associated with mammary development and lactation.

To date there have been no reports on the effects of grazing E+ perennial ryegrass on milk production in the ewe. Given that most ewe flocks in New Zealand lamb in early spring, neither endophyte toxin levels in the pasture nor ambient temperatures are likely to be high enough to affect mammary development. However, the effects of endophyte toxins and high ambient temperature on mammary development and subsequent lactation need to be examined in sheep to assess potential risks and extend the currently limited understanding of endophyte toxins in relation to milk production.

Therefore, the aims of this investigation were to 1): Examine the effects of exposure to ergovaline in late gestation on mammary development and the subsequent performance of the lamb and 2): Determine whether high ambient temperature exacerbates any effects ergovaline may have.

2. Materials and methods

2.1. Trial 1

2.1.1. Animals

On 10th June 1997, Forty, four-tooth Romney ewes were treated with an intra-vaginal progesterone (0.3 g) release device (EAZI-BREED CIDR-G, InterAg©). On 24th June the CIDR was removed and two Suffolk rams equipped with harness and crayons were introduced to the ewes. After one cycle length the crayon colour was changed and animals that returned to oestrus were removed from the group. The remaining ewes were examined by ultrasonography to confirm pregnancy and determine number of lambs carried by each ewe.

From this group, 18 single-bearing, pregnant ewes due to lamb 19th November 1997 were selected and allocated to one of two even groups.

2.1.2. Treatments

On 5th November 1997 the two groups of ewes were housed indoors in the cattle shed at the Animal Physiology Unit (APU), Massey University, Palmerston North.

Each ewe was kept in a 2.25 m^2 pen with *ad libitum* access to water (see Plate 7.1.). A 500 g diet of pelleted endophyte-free ryegrass seed and barley, supplemented with *ad libitum* access to chaffed pea vine hay (control diet) was fed for 5 days prior to the treatment period to allow the ewes to acclimatise to the diet and environment.

Ten days before the expected parturition date, one group of ewes (Ev+) had their pellets replaced with a 500 g ration of pelleted ryegrass seed, infected with an endophyte that produces ergovaline but not lolitrem B, and barley (ergovaline conc. of 10 ppm). The remaining group (Ev-) continued to receive the control diet.

The treatment diets continued until twenty days *postpartum* at which time all ewes were returned to outdoor grazing.

Plate 7.1. Housing arrangement for ewes

2.1.3. Sampling and measurements

Blood samples (5 ml) were taken daily at 0900 am by jugular venipuncture from two days prior to the start of the treatment period until 5 days after parturition and then every second day until the end of the treatment period.

After collection, blood samples were chilled on ice and centrifuged at 2500 rpm for 20 minutes. The serum fraction was taken off, and stored at - 20°C. The serum was analysed for prolactin.

Rectal temperature of each ewe was measured twice daily at 0800 am (pre-feeding) and 1500 pm (post-feeding) with a digital thermocouple thermometer.

After each ewe had lambed, the lambs were weighed daily and the ewes were milked on every 5th day *postpartum* to measure daily milk production. Milk samples were analysed for fat, lactose and protein concentrations.
The daily consumption of pellets, hay and water was also measured.

2.2. Trial 2

2.2.1. Animals

On 23rd January 1998, forty, six-tooth ewes were treated with a CIDR-G device as described in section 2.1.1. After CIDR-G withdrawal on 4th February, each ewe received 200 i.u. pregnant mare serum gonadotropin (PMSG, Folligon™) and two harnessed rams were introduced.

Ewes that conceived in the first mating were examined by ultrasonography 80 days after mating to confirm pregnancy and determine the number of foetuses carried per ewe.
From these, 20 single-bearing ewes were selected and divided into four even groups with an expected lambing date of the 4th July 1998.

2.2.2. Treatments

On 10th June 1998, the four groups of ewes were housed in controlled temperature rooms at the APU. Two groups were kept at 32°C (high temperature) and the remaining two groups were kept at 18°C (low temperature). Each ewe was contained in a 2.25 m^2 pen with *ad libitum* access to water. The lighting in the rooms was automatically controlled to 12 hours light (0600 am to 1800 pm) and 12 hours dark each day.

All ewes received a diet of 500 g pelleted endophyte-free ryegrass seed and barley supplemented with 500 g chaffed pea vine hay (control diet) for five days prior to the treatment period to allow the animals to acclimatise to the diet and environment. From day twenty before the expected lambing date, one group of ewes in each room had their pellets replaced with a 500 g ration of pelleted endophyte-infected ryegrass seed (as in trial 1) and barley (ergovaline conc. 10 ppm). This diet was maintained until day twenty *postpartum.* The remaining group (Ev-) continued to receive the control diet during this time. Once the ewes had lambed the daily allowance of chaffed hay was increased to 1500 grams.

Two ewes were removed from the Ev+ low temperature treatment, and one ewe was removed Ev- low temperature treatment when they refused to consume the pelleted diet. This may have been due to acidosis caused by consuming the concentrate diet.

2.2.3. Sampling and measurements

All ewes were blood sampled by jugular venipuncture, and rectal temperatures taken pre-treatment, and subsequently every second day at 0900 am. Blood samples and rectal temperatures were taken daily between day five *prepartum* and day five *postpartum.* Blood samples were handled in the same manner described in section 2.1.3. Serum samples were assayed for serum prolactin, progesterone, cortisol, and insulin.

On the expected lambing date, mammary gland girth and teat length was measured using string marked with graduations, and recorded photographically.
Lambs were weighed daily from birth to determine growth rate.

Daily fluctuations in ambient temperature and humidity were recorded every 15 minutes for three days during the trial with a digital data logger (HOBO™, provided by John Ellis, Hort+Research, Palmerston North) and downloaded onto a computer. This showed that the desired ambient temperature in each room did not change significantly. Relative humidity was significantly higher in the low temperature room and increased significantly in the high temperature room after the room was washed down each morning. Daily changes in temperature and relative humidity in the high and low temperature rooms are shown in appendix II.

2.3. Faecal moisture

At the conclusion of the trial, two Ev- ewes in the low temperature room were kept in their pens for a further 10 days. Each ewe received the control diet for two days after which time one of the ewes was given the Ev+ diet for two days while the remaining ewe continued to receive the Ev- diet. After this second two-day period the diets were switched between the ewes and fed for the remaining six days. Faecal grab samples were collected from the rectum of each ewe, weighed and dried to determine faecal moisture content.

2.4. Serum prolactin analysis

Serum prolactin levels were determined using a double-antibody ^{125}I radioimmunoassy procedure based on the methods of Van Landegham & Van de Weil (1978) at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand (see Chapter VI, section 2.3.2. for details).

2.5. Serum progesterone analysis

Serum progesterone levels were determined using a solid-phase ^{125}I radioimmunoassy kit (Coat-A-Count ®, Diagnostic Products Corporation, Los Angeles, CA) in accordance with the manufacturer's instructions, at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand (see Chapter VI, section 2.3.1. for details).

2.6. Serum cortisol analysis

Serum cortisol levels were determined using a solid phase ^{125}I radioimmunoassy kit (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA) in accordance with the manufacturer's instructions, at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand (Procedure was similar to that described for progesterone analysis in Chapter VI, section 2.3.1. for details).

The inter-assay and intra-assay co-efficient of variation was 5.2% and 4.1% respectively

2.7. Serum insulin analysis

Serum insulin levels were determined using a double-antibody ^{131}I radioimmunoassy procedure based on the methods of Flux *et al.* (1984) at the Institute of Food and Human Health, Massey University, Palmerston North, New Zealand. Bovine insulin (I-1550, Sigma Chemical Company, St Louis, MO) was used for the standards in this assay.

The inter-assay and intra-assay co-efficient of variation was 13.5% and 8.5% respectively

2.8. Statistical analyses

All statistical analyses were carried out using Graph-pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.

Serum hormone levels, rectal temperature, milk constituents, feed and water intake and lamb growth rate data were analysed by two-way analysis of variance (ANOVA) to determine variation due to ergovaline (treatment), day of treatment (time) and treatment X time interactions. Mammary gland measurements were analysed by t-test. All data are reported as means $\pm$ standard error of the mean (SEM).

Data was analysed for only 3 ewes in the Ev- low temperature group and 4 ewes in the Ev- low temperature group for reasons described in section 2.2.2.

3. Results

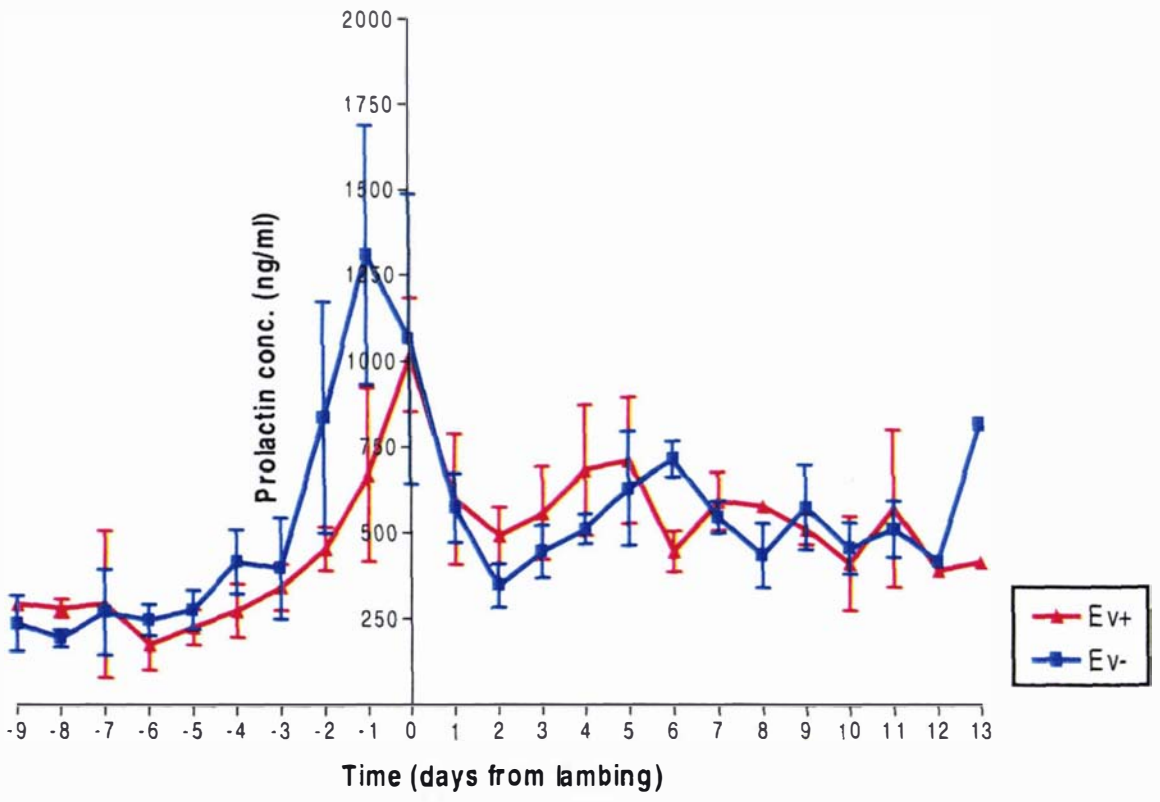
3.1. Trial 1

3.1.1. Serum prolactin

Mean serum prolactin concentration in the Ev+ and Ev- ewes in Trial 1 are shown in Figure 7.1.

Serum prolactin profiles, prior to and after parturition were not significantly distinct between the treatment groups. Serum prolactin increased significantly ($P<0.001$) in both groups from –3 days to lambing and then decreased significantly ($P<0.001$) from lambing to day 2. There was no significant interaction between day from lambing and treatment.

Figure 7.1. Mean (±SEM) serum prolactin concentration in ewes feed diets either with or without ergovaline (Trial 1).



3.1.2. Milk constituents

Milk constituent data for Trial 1 are summarised in Table 7.1.

There were no significant differences in milk fat, protein or milk solids between the Ev+ and Ev- group. Milk lactose levels were significantly ($P<0.05$) higher in the Ev- group on days 15 and 20 of lactation. There was no significant effect of day of lactation on any milk constituent and no interaction between day of lactation and treatment.

There were no significant differences in milk volume between the Ev+ and Ev- groups or between the days of lactation sampled.

Table 7.1. Mean (±SEM)% milk fat, protein, lactose and total milksolids at days 5, 10, 15 and 20 of lactation in ewes fed a diet with (Ev+) or without (Ev-) ergovaline.

	FAT		PROTEIN		LACTOSE		TOTAL SOLIDS	
Day of lact.	**Ev+**	**Ev-**	**Ev+**	**Ev-**	**Ev+**	**Ev-**	**Ev+**	**Ev-**
5	8.60 ± 0.51	9.33 ± 0.69	4.30 ± 1.36	5.26 ± 0.33	5.14 ± 0.12	5.19 ± 0.15	19.29 ± 0.52	19.78 ± 0.66
10	8.73 ± 0.61	9.38 ± 0.89	5.11 ± 0.16	4.94 ± 0.21	5.17 ± 0.18	5.33 ± 0.061	19.01 ± 0.55	19.65 ± 0.86
15	8.63 ± 0.46	8.22 ± 0.75	4.83 ± 0.17	4.58 ± 0.04	5.23 ± 0.09	5.45 ± 0.08[a]	18.70 ± 0.54	18.25 ± 0.67
20	7.93 ± 0.19	8.76 ± 0.52	4.64 ± 0.13	4.77 ± 0.15	5.24 ± 0.16	5.52 ± 0.07[a]	17.81 ± 0.16	19.04 ± 0.41

[a] means differed significantly between treatments ($P<0.05$).

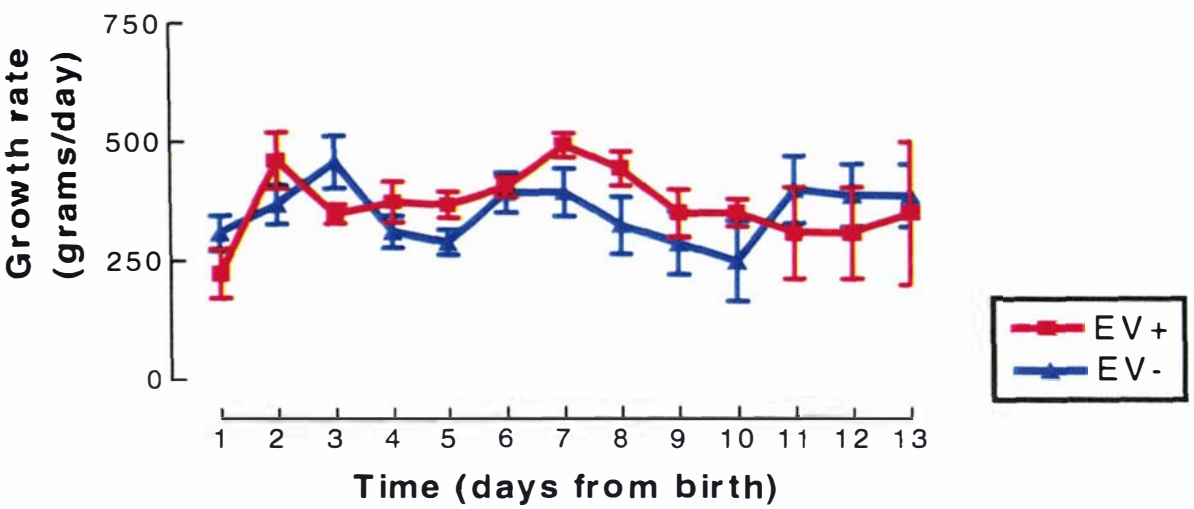
3.1.3. Birthweight and lamb growth rate

There were no significant differences in lamb birthweight between treatment groups. The mean (±SEM) birth weights were 4.98 ± 0.65 kg and 5.23 ± 0.38 kg for the Ev+ and Ev- groups respectively.

Mean daily growth rates of lambs in the Ev+ and Ev- groups are shown in Figure 7.2.

There were no significant differences in daily growth rate between lambs in the Ev+ and Ev- groups. There was no significant effect of day after birth or interaction between day after birth and treatment.

Figure 7.2. Mean (±SEM) daily growth rate of lambs born to ewes with (Ev+) or without (Ev-) ergovaline in their diet (Trial 1).

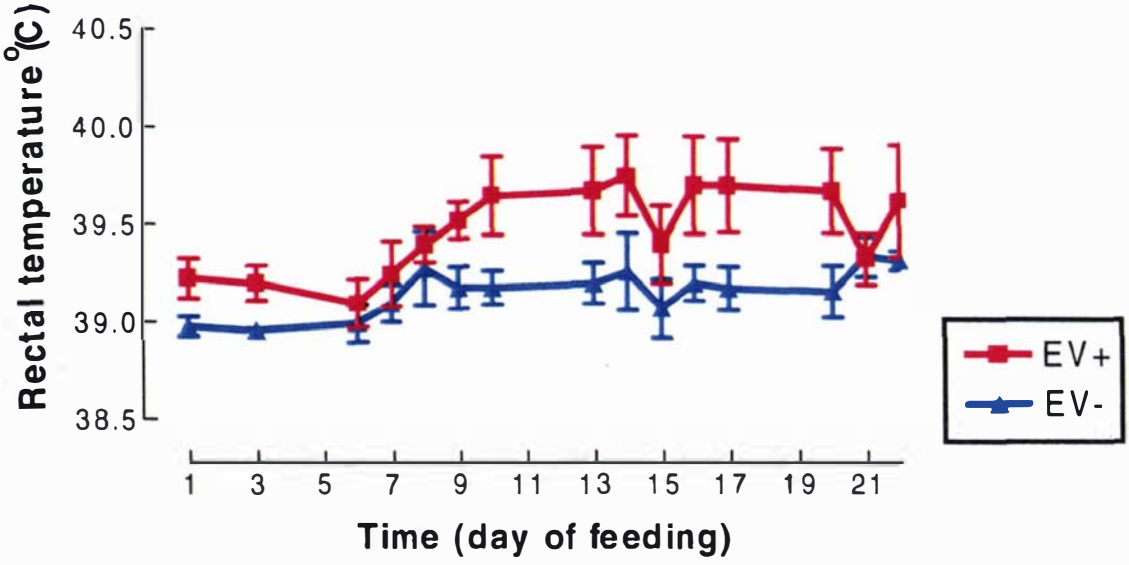


3.1.4. Ewe rectal temperature

Mean rectal temperatures for Ev+ and Ev- ewes are shown in Figure 7.3.

Rectal temperature was significantly ($P<0.001$) higher in the Ev+ ewes than in the Ev- ewes. Rectal temperature increased significantly ($P<0.05$) in both groups after parturition. There was no significant interaction between day of measurement and treatment.

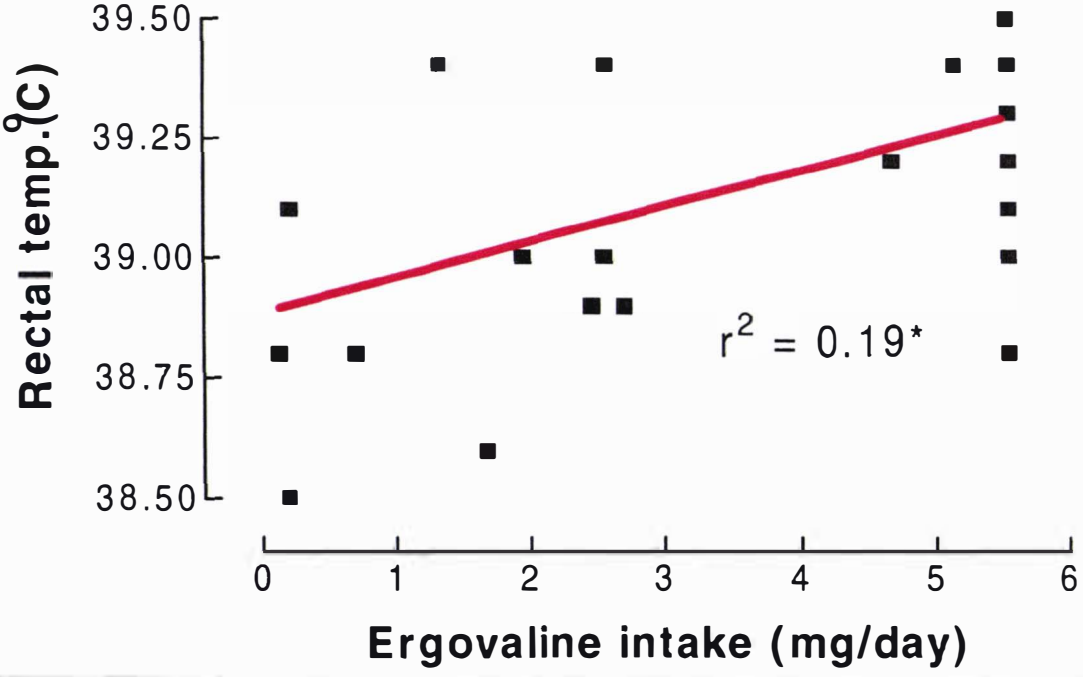
Figure 7.3. Mean (±SEM) rectal temperatures in ewes offered diets with (Ev+) of without (Ev-) ergovaline (Trial 1).



3.1.5. Relationship between rectal temperature and daily ergovaline intake

There was a significant (P<0.01) positive linear relationship between daily ergovaline intake and rectal temperature (see Figure 7.4.)

Figure 7.4. Relationship between daily ergovaline intake and rectal temperature in ewes (Trial 1).

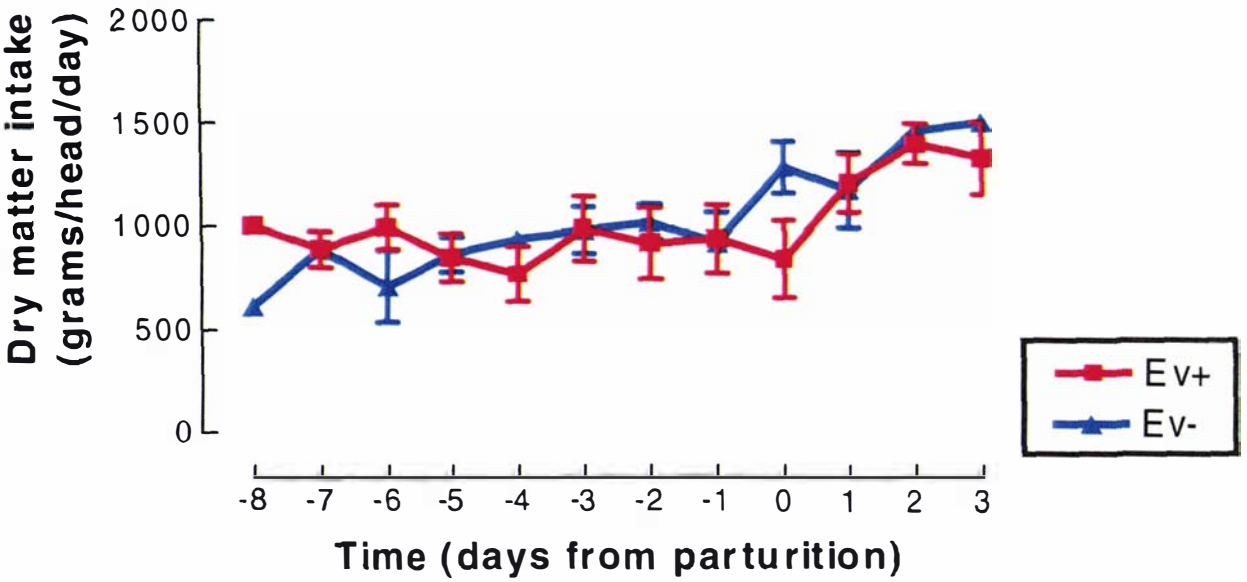


3.1.6. Feed intake

Mean (±SEM) daily total dry matter intakes for the Ev+ and Ev- groups relative to day from parturition are shown in Figure 7.5.

There was no significant difference in pellet, hay or total dry matter consumption between the Ev+ and Ev- groups. Daily total dry matter consumption increased significantly (P<0.0001) post-parturition in both groups due to a significant increase in hay dry matter intake.

Figure 7.5. Mean (±SEM) daily total dry matter intake of ewes fed diets with (Ev+) or without (Ev-) ergovaline during late pregnancy (Trial 1)

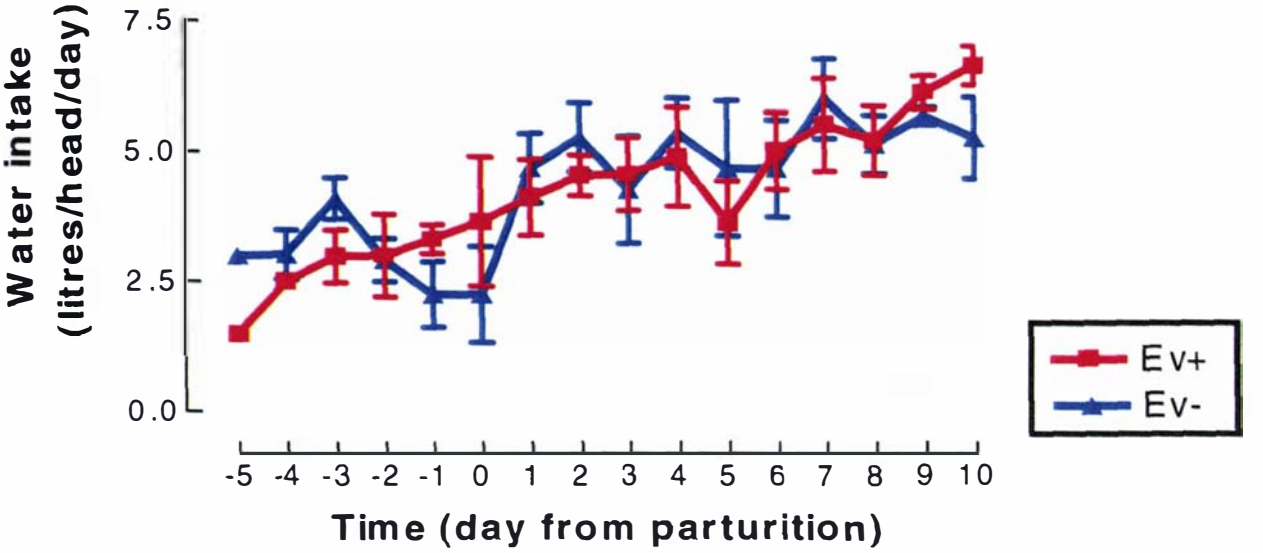


3.1.7. Water intake

Mean (±SEM) daily water consumption for the Ev+ and Ev- groups relative to day from parturition are shown in Figure 7.6.

There was no significant difference in daily water consumption between the Ev+ and Ev- groups. Ewe daily water consumption increased significantly (P<0.0001) post parturition.

Figure 7.6. Mean (±SEM) daily water consumption of ewes fed diets with (Ev+) or without (Ev-) ergovaline during late pregnancy (Trial 1).



3.2. Trial 2

3.2.1. Serum prolactin

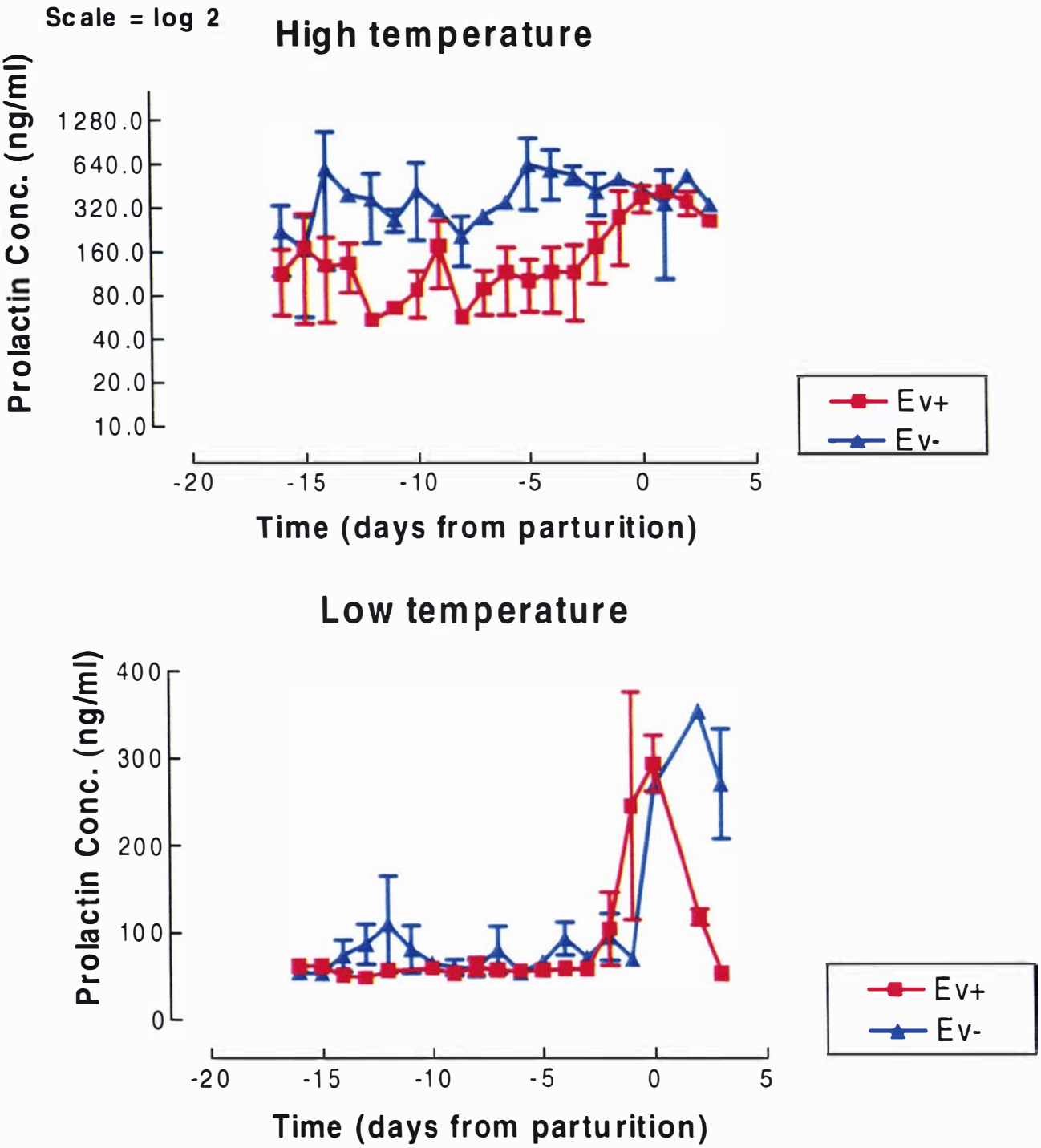
Serum prolactin levels in the Ev+ and Ev- ewes in the high and low temperature rooms are shown in Figure 7.7.

There was no significant difference in serum prolactin levels between ewes on the ergovaline diet and those on the ergovaline-free diet in the low ambient temperature treatment. There was a significant ($P<0.05$) rise in serum prolactin one day before parturition in both groups of ewes in the low temperature treatment.

In the high temperature treatment, serum prolactin levels were significantly ($P<0.0001$) lower in the ewes on the ergovaline diet than in ewes on the ergovaline-free diet. There was no significant effect of day from parturition on serum prolactin levels in the high temperature groups.

There was an effect of ambient temperature on prolactin levels with the Ev+ and Ev- ewes in the high temperature room having significantly ($P<0.001$) higher serum prolactin levels than the respective groups in the low temperature room. There was a significant ($P<0.001$) interaction between ambient temperature and treatment.

Figure 7.7. Mean (±SEM) serum prolactin levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline and high and low ambient temperature (Trial 2).



3.2.2. Serum progesterone

Serum progesterone levels in the Ev+ and Ev- ewes in the high and low temperature rooms are shown in Figure 7.8.

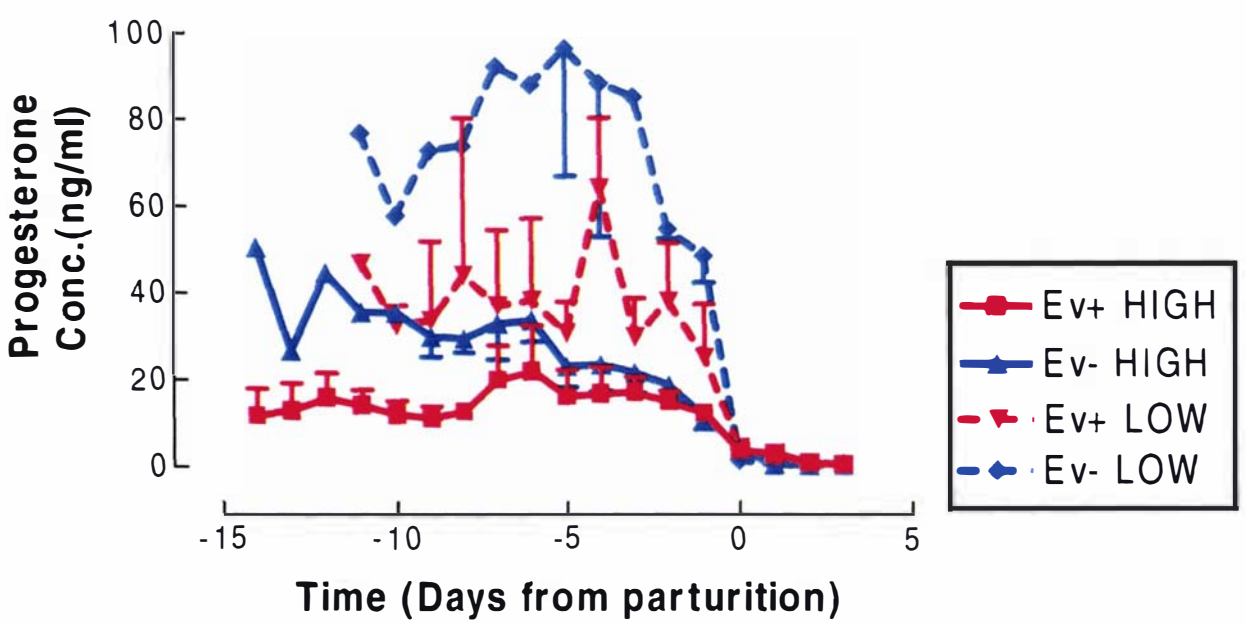
Serum progesterone levels reported for the ewes appeared to be 10-fold higher than levels considered within the normal range for ewes at this stage of pregnancy. This may have been due to an error in calculation of the progesterone concentrations from the raw assay data. However, the comparisons made are still valid, as the relative differences in progesterone between the treatments were unaffected.

Serum progesterone levels were significantly ($P<0.001$) lower in ewes fed the ergovaline diet than in those fed the ergovaline-free diet in the low ambient temperature treatment.

In the high temperature treatment, serum progesterone levels were significantly ($P<0.0001$) lower in the ewes on the ergovaline diet than in those on the ergovaline-free diet. There was a significant ($P<0.001$) fall in serum progesterone one day before parturition in all groups of ewes.

There was an effect of ambient temperature on progesterone levels with the Ev+ and Ev- ewes in the high temperature room having significantly ($P<0.001$) lower serum progesterone levels than the respective groups in the low temperature room. There was a significant ($P<0.001$) interaction between ambient temperature and treatment.

Figure 7.8. Mean (±SEM) serum progesterone levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline and high and low ambient temperature (Trial 2).

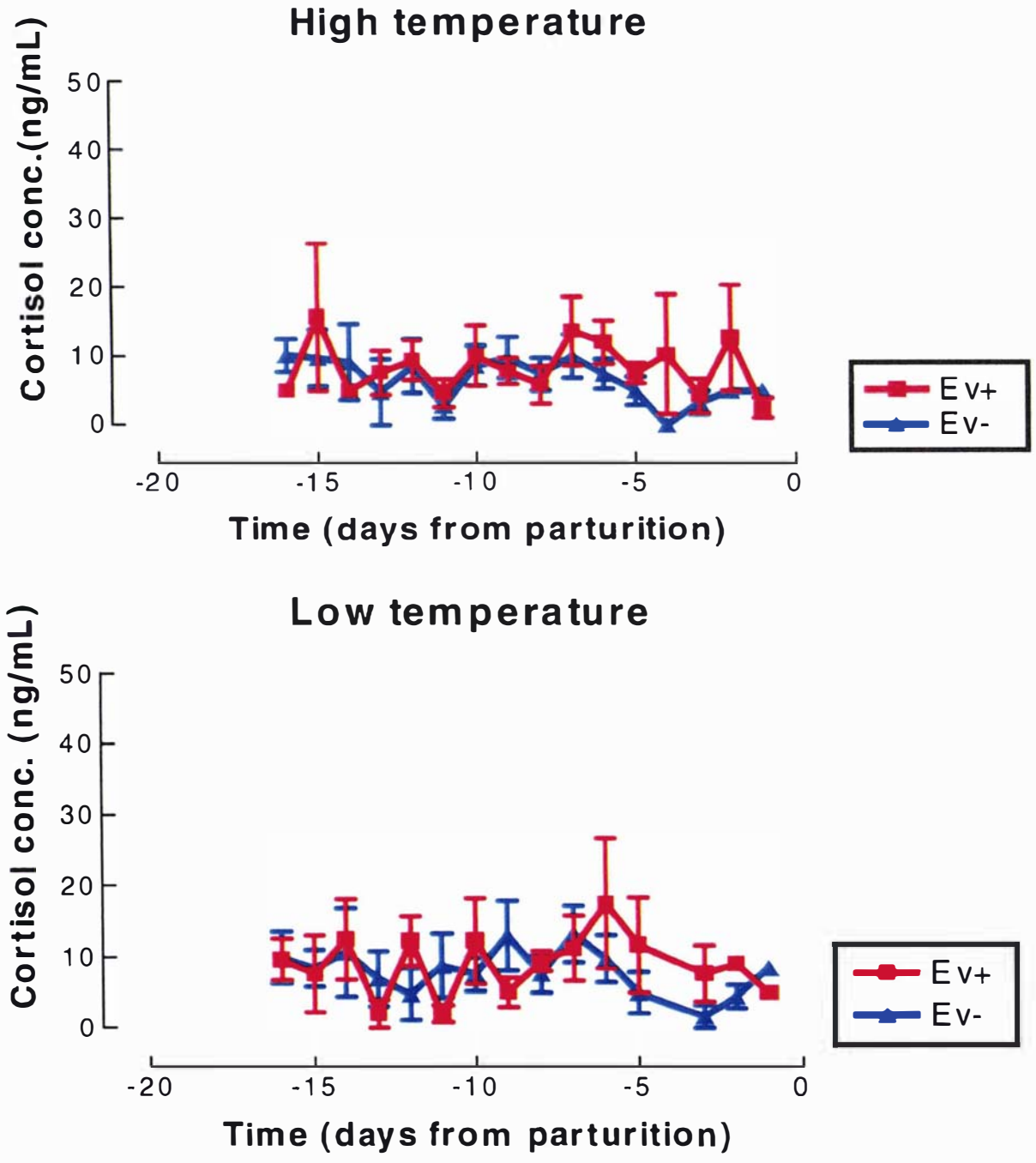


3.2.3. Serum cortisol

Serum cortisol levels in the Ev+ and Ev- ewes in the high and low temperature rooms are shown in Figure 7.9.

There were no significant differences in serum cortisol levels in any of the treatment groups and levels were in the expected range for ewes at that stage of pregnancy.

Figure 7.9. Mean (±SEM) serum cortisol levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline at high and low ambient temperature (Trial 2).



3.2.4. Serum insulin

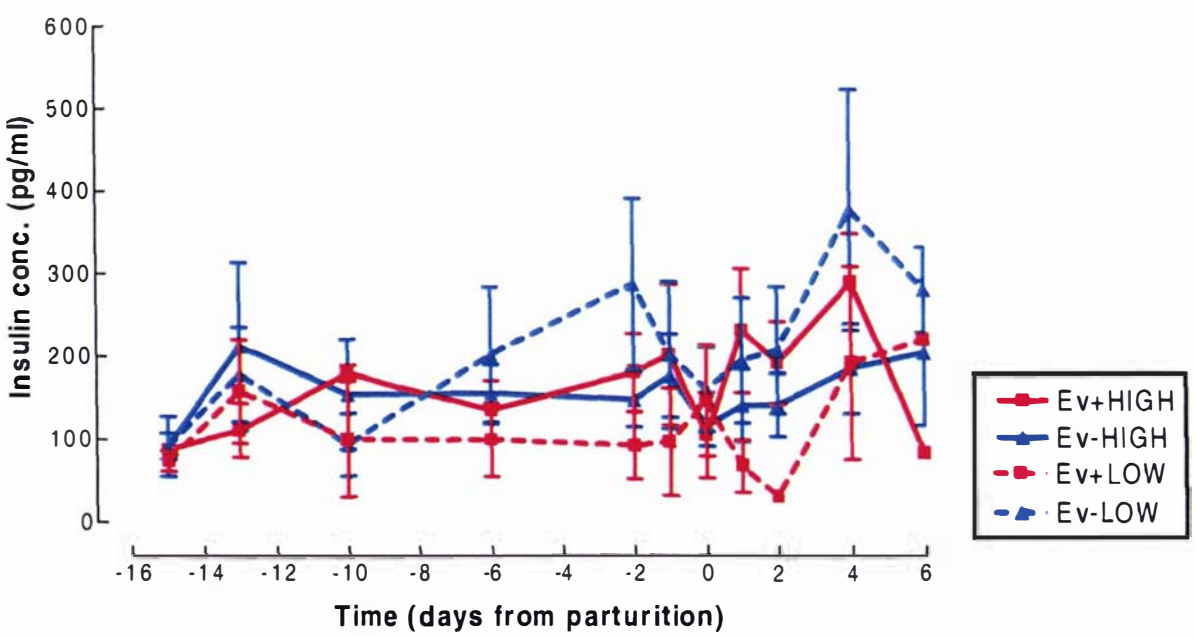
Serum insulin levels in the Ev+ and Ev- ewes in the high and low temperature rooms are shown in Figure 7.10.

There were no significant differences in serum insulin levels between the Ev+ and Ev- ewes in the high temperature room during the trial period. Ev+ ewes in the low temperature room had significantly ($P<0.01$) lower serum insulin levels than the Ev- ewes in the low temperature room from 6 days before parturition to 6 days after parturition. High ambient temperature significantly ($P<0.1$) reduced serum insulin levels in ewes feed the Ev- diet, however, there was no significant difference in the Ev+ ewes between the low and high temperature treatments.

There were no significant diet x time, or temperature x time interactions.

Serum insulin levels fell in the Ev+ high, Ev- high and the Ev- low groups at parturition and then increased after parturition to levels greater than those pre-parturition. Serum insulin levels in the Ev+ low group increased slightly at parturition and then fell during the 2 days after parturition to pre-parturition levels before increasing again.

Figure 7.10. Mean (±SEM) serum insulin levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline at high and low ambient temperature (Trial 2).



3.2.5. Rectal temperature

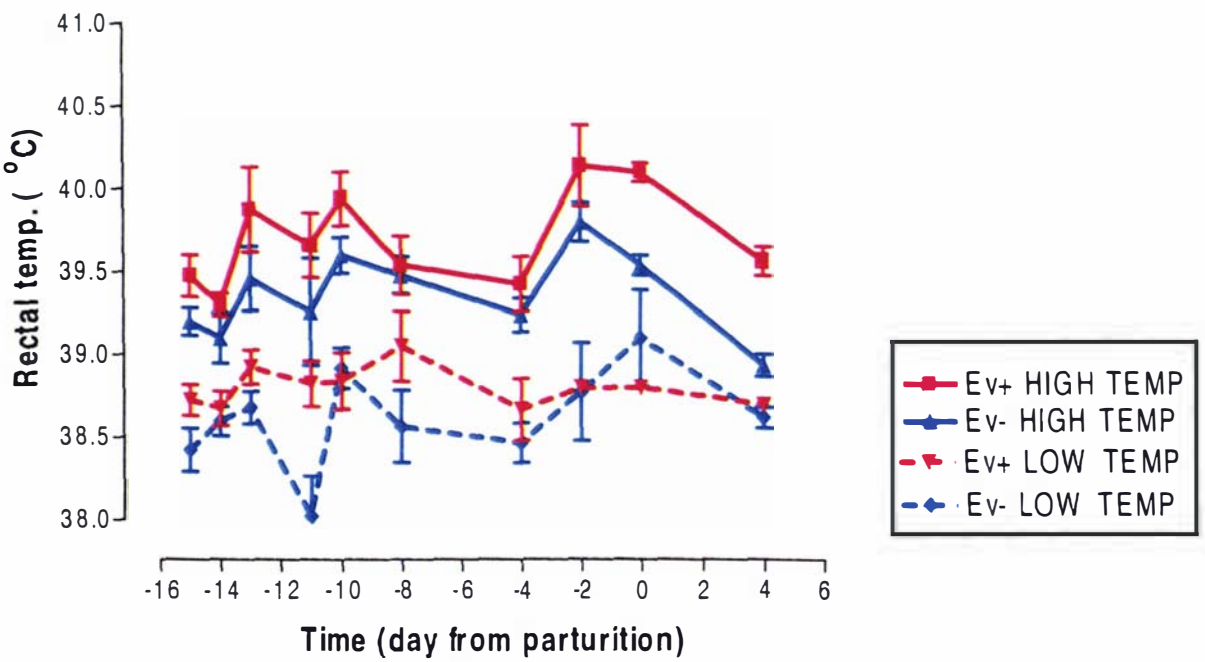
Mean rectal temperature for the groups of ewes are shown in Figure 7.11.

There was no significant difference in rectal temperature between the Ev+ and Ev- ewes in the low temperature room. Rectal temperature was significantly ($P<0.0001$) higher in the Ev+ ewes than the Ev- ewes in the high temperature room.

There was a significant ($P<0.05$) time effect on rectal temperature in all groups of ewes with rectal temperatures increasing close to, and during parturition.

There was no significant treatment X time interaction, however, there was a significant ($P < 0.001$) treatment by environmental temperature interaction.

Figure 7.11. Mean (±SEM) rectal temperature in ewes fed a diet with (Ev+) or without (Ev-) ergovaline at high and low ambient temperature (Trial 2).



3.2.6. Milk production and mammary development

All Ev+ ewes in the high temperature room were agalactic and showed very little mammary development.

There was significantly ($P<0.05$) greater mammary development, determined by mammary gland girth measurement, in the Ev- ewes than in the Ev+ ewes in the high temperature room (25.0 $\pm$ 1.5 cm and 17.6 $\pm$ 1.9 cm for the Ev- and Ev+ ewes respectively). There was no significant difference in mammary gland development between the Ev+ and Ev- ewes in the low temperature room (20.5 $\pm$ 2.5 cm and 27.5 $\pm$ 3.2 cm for the Ev- and Ev+ ewes respectively), however, Ev+ ewes were reluctant to allow lambs to suckle.

Plates 7.2., 7.3., 7.4., and 7.5. show the mammary glands of a typical ewe from the Ev+ and Ev- low temperature and the Ev+ and Ev- high temperature groups respectively.

Due to the agalactia in the Ev+ high temperature group and the reluctance to nurse in the Ev+ low temperature group milk production was not measured.

3.2.7. Lamb growth rate

All lambs born to ewes in the Ev+ high temperature group and many of the lambs born to ewes in the Ev+ low temperature group, required hand rearing and, therefore, lamb weighing was discontinued.

3.2.8. Maternal behaviour

In ewes fed the diet containing ergovaline there appeared to be a reluctance to allow the lamb to suckle. This reluctance often led to aggression by the ewe towards its own lamb. In the high temperature treatment this may have been exacerbated by the lack of milk and weak lambs. However, this change in normal maternal behaviour occurred in the low temperature treatment where mammary development appeared to be normal.

3.2.9. Faecal moisture

Faecal moisture of ewes that were fed the Ev+ or Ev- diets for two days and then the opposite diet for a further six days are shown in Figure 7.12.

When the diet was changed from Ev- to Ev+ faecal moisture increased in both ewes from 60% to more than 80% in ewe 1 and 75% in ewe 2. When the diet was switched back to Ev- in ewe 1 the faecal moisture level fell but did not return to pre Ev+ diet levels within the following six-day period.

Figure 7.12. Faecal moisture in a ewe that was fed Ev+ diet then the Ev- diet (ewe 1) and a ewe that was fed the Ev- diet then the Ev+ diet (ewe 2)

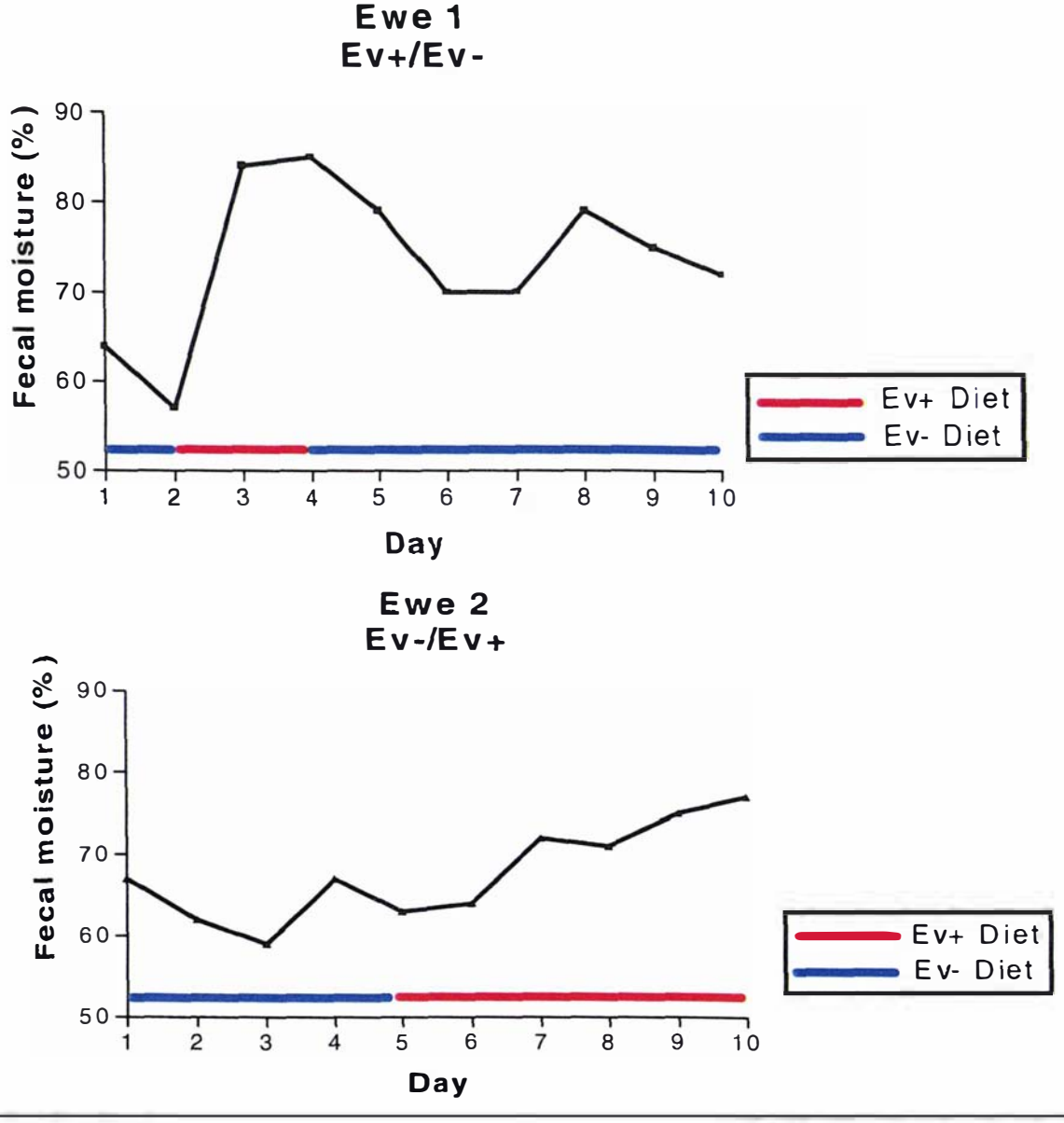


Plate 7.2. Mammary gland of Ev+ low temperature ewe

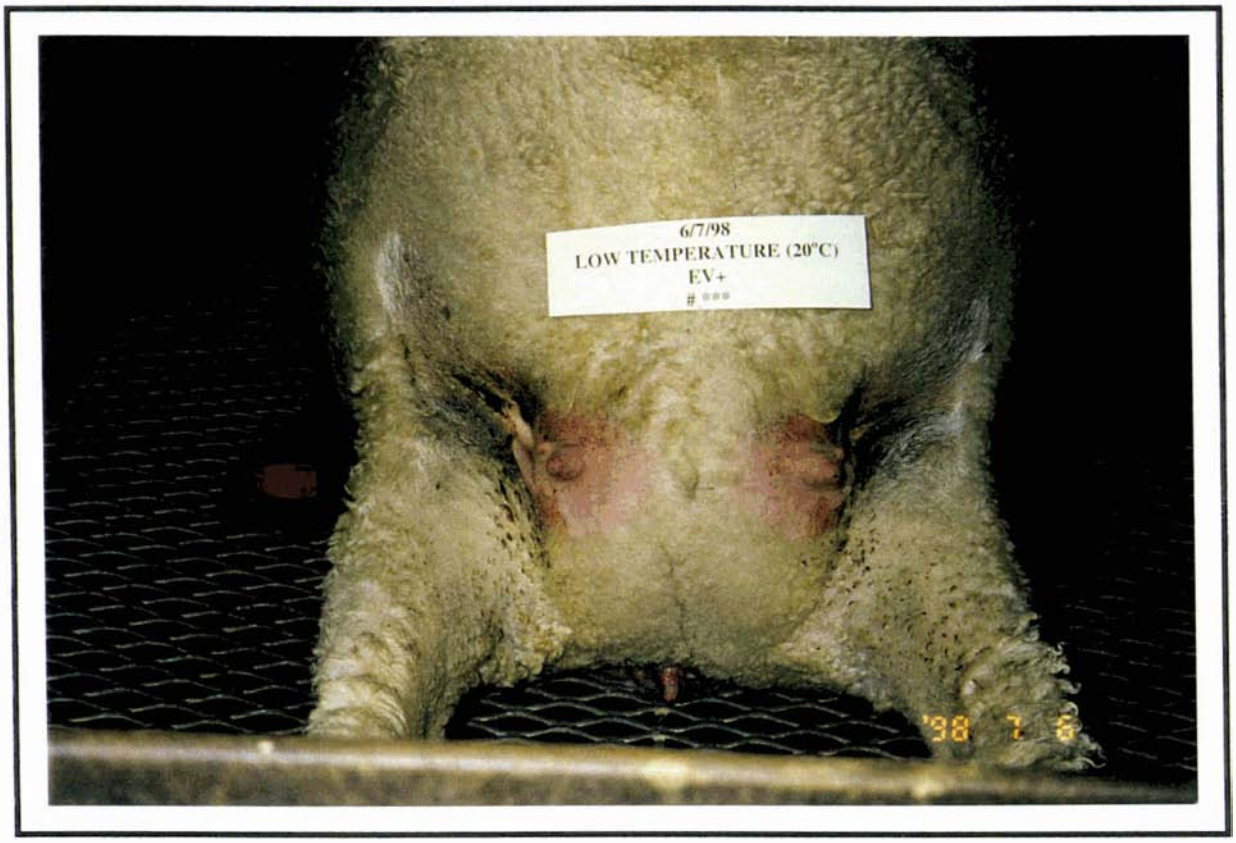


Plate 7.3. Mammary gland of Ev- low temperature ewe

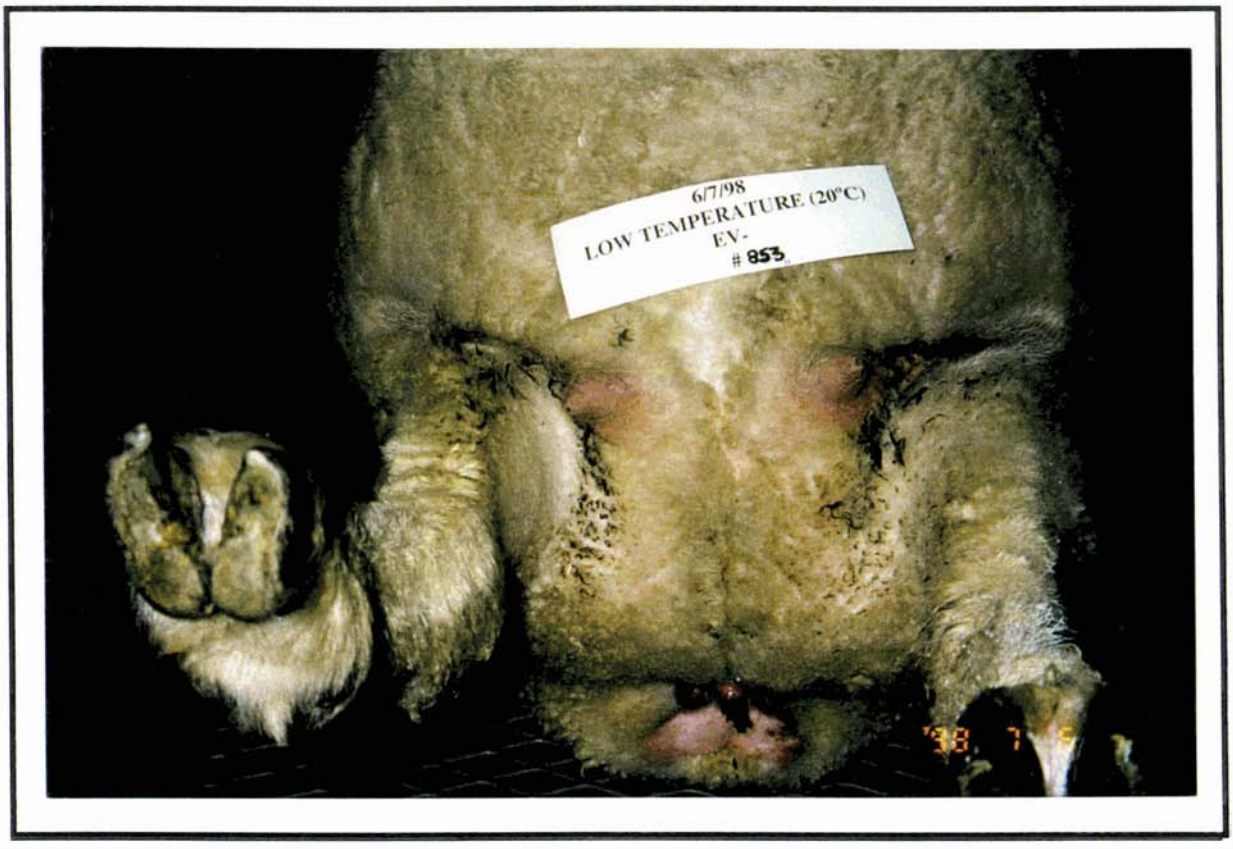


Plate 7.4. Mammary gland of Ev+ high temperature ewe

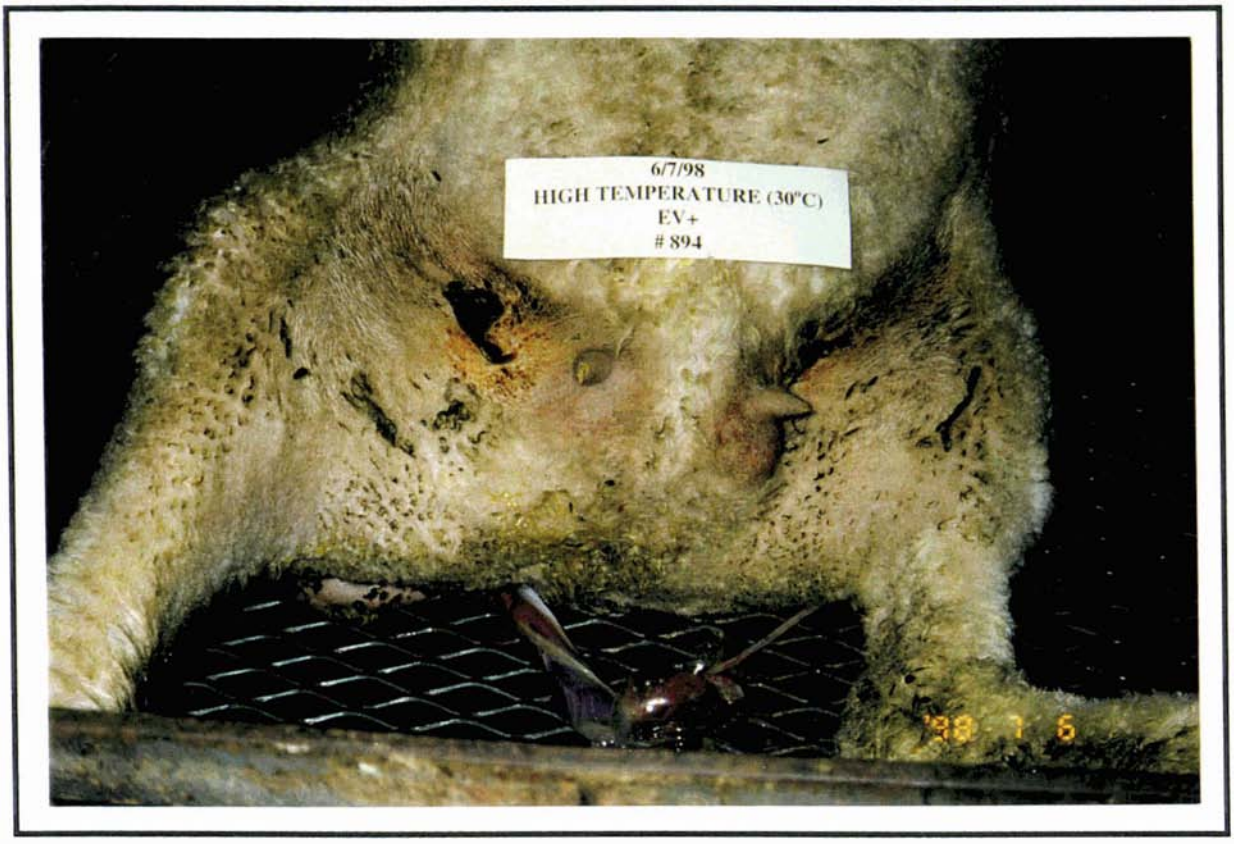
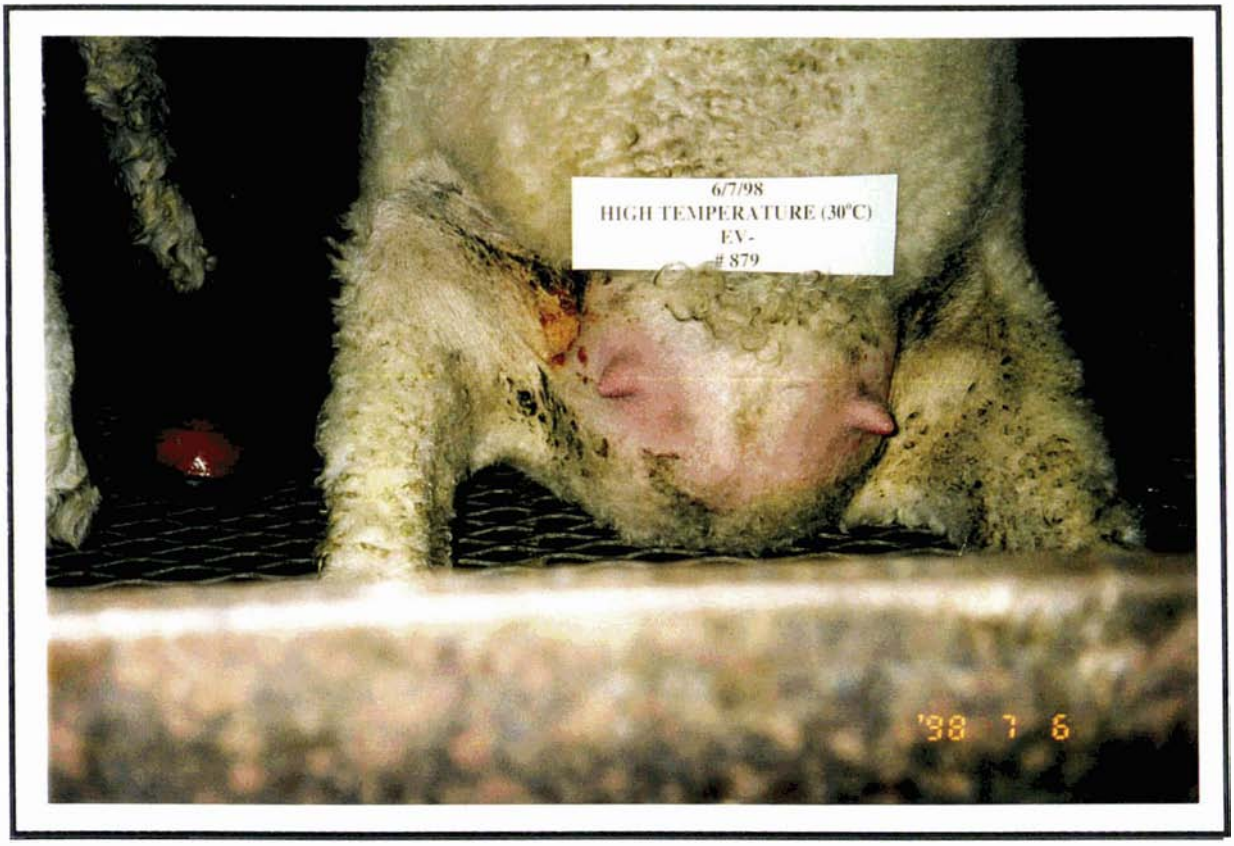


Plate 7.5. Mammary gland of Ev- high temperature ewe



4. Discussion and conclusions

4.1. Serum prolactin

Serum prolactin levels were not significantly affected by ergovaline at thermoneutral temperatures in Trial 1 and in the low temperature room in Trial 2, but were significantly lower in the Ev+ ewes than in the Ev- ewes under conditions of high ambient temperature.

There was a large significant effect of ambient temperature on serum prolactin levels and a significant interaction between ergovaline and ambient temperature. A positive relationship between high ambient temperature and increased prolactin secretion in sheep has been reported (Hill & Alliston, 1981; Fletcher *et al.*, 1997), and results in the current trials are a further indication of this.
The role of prolactin in thermoregulation is discussed further in section 4.6.

Serum prolactin levels in sheep normally rise rapidly in the last 2-3 days of pregnancy to peak around the time of parturition, followed by a decline during early lactation to levels higher than those characteristic of late pregnancy before the *prepartum* surge (Gow *et al.*, 1983; Mellor *et al.*, 1987). This prolactin secretion profile was clearly exhibited in both the Ev+ and Ev- ewes in Trial 1 and in the low temperature room in Trial 2. Despite the suppressive effects of ergovaline in the high temperature room the normal *prepartum* rise in serum prolactin was not completely abolished in the Ev+ ewes. However, no *prepartum* rise in serum prolactin was exhibited in the Ev- ewes in the high temperature room. This may have been due to the fact that serum prolactin levels were already as high as *prepartum* peak levels due to the high ambient temperature.

Prepartum serum prolactin levels in the ewes in Trial 1 were significantly ($P<0.05$) higher in both groups of ewes than they were in either group in the low temperature room in Trial 2. This difference between trials could not be attributed to differences in the duration of the ergovaline treatment, as the differences in prolactin between the trials were evident in both the Ev+ and Ev- ewes. The differences in serum prolactin may have been due to differences in the light:dark ratio between the trials. Longer photoperiod has been shown to increase prolactin levels in both pregnant and lactating ewes (Munro *et al.*, 1980). Ewes in Trial 1 were exposed

to a longer photoperiod than the ewes in Trial 2 and this may have increased prolactin secretion.

The depression of serum prolactin levels in animals grazing E+ pasture or dosed with ergopeptine alkaloids is one of the most consistent effects. Decreased milk production has been reported in horses, cattle and sheep grazing E+ pasture and it is possible that the depression of prolactin might contribute to this.

All reports to date on serum prolactin levels in sheep fed diets containing endophyte toxins have been with non-pregnant animals. Some studies showed significant suppression of prolactin secretion in pregnant ewes injected with the synthetic ergot alkaloid, bromocriptine (CB154) (Kann, 1976a; 1976b; Peterson *et al.,* 1997). However, in these trials high doses of the ergot alkaloid were injected directly into the animal rather than incorporated into the diet as in the current trial.

The current trials were the first to examine serum prolactin levels in sheep fed a diet containing ergovaline prior to parturition.

4.2. Serum progesterone

Ergovaline and ambient temperature significantly affected serum progesterone levels. Serum progesterone was significantly lower in ewes fed diets containing ergovaline than in ewes fed ergovaline-free diets in both the high and low temperature rooms. This is in contrast to the findings of Peterson *et al.* (1997) who showed no significant differences in serum progesterone between ewes that were injected daily with the synthetic ergot alkaloid CB154 from 20 days *prepartum* to parturition and ewes that were untreated. Serum progesterone levels were not measured in Trial 1.

Lower serum progesterone levels have been reported in pregnant mares grazing endophyte-infected tall fescue (Monroe *et al.,* 1988). However, the effects of endophyte on serum progesterone in other species of grazing animal have either been variable or have not been investigated (the case with sheep). Results in the current trial appear to support evidence that

endophyte may reduce serum progesterone in pregnant animals although the mechanisms by which this occurs are not known.

High ambient temperature suppressed serum progesterone. High ambient temperatures are known to decrease serum progesterone in sheep (Berman, 1991; Hill, 1980) and results in Trial 2 support this. There was also a significant interaction between ergovaline and ambient temperature. Differences in serum progesterone were less between the Ev+ and Ev- groups in the high temperature room, where progesterone was also suppressed by the high temperature, than between Ev+ and Ev- groups in the low temperature room. However, the greatest suppression of serum progesterone was observed in the Ev+ group in the high temperature room.

Although ergovaline and high ambient temperature significantly affected the levels of serum progesterone, the general profile of progesterone secretion did not appear to be affected. Serum progesterone profiles during the last 10 days of pregnancy are similar to other reports on sheep during this period (Bassett, 1969; Chamley *et al.,* 1973) with peak progesterone levels reached between 5 and 10 days *prepartum* and a sharp decline from 5 days *prepartum* to parturition. Serum progesterone levels in the Ev- ewes in the low temperature room were significantly higher than levels reported in any of these studies for single-rearing ewes at the same stage of pregnancy, however, wide variation in serum progesterone concentrations between individual pregnant ewes is common (Slotin *et al.,* 1971).

Progesterone has a major role in the maintenance of pregnancy and may have a role in growth and development of the mammary gland. Progesterone also has an important role in lactogenesis. The falling progesterone levels at the end of pregnancy permit the stimulation of α-lactoglobulin synthesis by prolactin, completing the lactose synthetase unit, and so the catalysis of the final step in lactose synthesis. (Cowie & Tindal, 1971). Differences in progesterone secretion may have been responsible for the significant differences in milk lactose levels between the Ev+ and Ev- ewes in Trial 1, although this cannot be verified. It is possible that impairment of progesterone secretion by ergovaline could have significant effects on both pregnancy and mammary development. It is also likely that high ambient temperatures will exacerbate any of these effects. It could be that increased progesterone may delay or reduce lactogenesis in hotter conditions.

4.3. Serum cortisol

Ergovaline and ambient temperature did not appear to significantly affect serum cortisol levels in pregnant ewes in Trial 2 (cortisol levels were not measured in Trial 1). Serum cortisol levels in all groups of ewes were within the normal range for ewes at that stage of gestation (Mellor *et al.,* 1987).

This is the first investigation in which serum cortisol levels have been examined in pregnant ewes given diets containing endophyte toxins and there are very few reports on serum cortisol levels in other species of animal either dosed with endophyte toxins or grazing E+ pasture. Browning *et al.* (1998) found that the ergopeptine alkaloid ergotamine tartarate, which is similar to ergovaline, increased serum cortisol levels in steers. However, others have failed to find any difference in serum cortisol between animals grazing E+ and E- pasture.

4.4. Serum insulin

Serum insulin levels were significantly lower in ewes given the Ev+ diet in the low temperature room than ewes given the Ev- diet. However, there were no significant differences in serum insulin levels between the Ev+ and Ev- ewes in the high temperature room. Serum insulin levels in the high temperature room may have been suppressed by the temperature as indicated by the difference in insulin levels in the Ev- ewes between the high and low temperature rooms. Therefore, the suppressive effects of high ambient temperature on serum insulin may have masked any effects of ergovaline.

Serum insulin profiles were similar to other observations in ewes at late pregnancy and parturition (Mellor *et al.,* 1987; Peterson *et al.,* 1997) with an increase in insulin levels directly after parturition. The post-parturient rise in insulin levels appeared to be delayed in the Ev+ low temperature group compared with the other groups. This rise in insulin levels after parturition is probably due to the rise in plasma glucose at this time, however, other factors are also involved (Mellor *et al.,* 1987). Serum glucose levels were not measured in the present trial so this cannot be verified as the cause of the increase in insulin levels or of the delayed insulin increase in the Ev+ low temperature group.

To date, serum insulin levels have not been compared between sheep fed diets with or without ergovaline, nor have they been compared in sheep grazing either endophyte-infected or endophyte-free pastures. Some researchers found that serum insulin levels in cattle were not affected by grazing E+ tall fescue (Thompson *et al.*, 1987; Harmon *et al.*, 1991).

The effects of the ergot alkaloid bromocriptine on serum insulin levels in sheep have been examined, however, they are generally inconclusive. Johansson *et al.* (1986) found that daily bromocriptine injections reduced serum insulin levels in female lambs compared with untreated lambs. Peterson *et al.* (1997) found that daily bromocriptine injections in ewes for twenty days *prepartum* increased serum insulin levels compared to control animals whereas a nine day *prepartum* bromocriptine treatment reduced serum insulin relative to the control ewes. The reasons for this could not be logically explained by the authors. Similar studies with bromocriptine in lactating goats failed to find any significant affect on serum insulin levels (Forsyth & Lee, 1993; Mahendra-Singh *et al.*, 1999).

The variable results in previous reports make interpretation of the results obtained in the present trial difficult, however, it appears that ergovaline does suppress serum insulin levels.

There was a significant effect of ambient temperature on serum insulin levels only in the absence of ergovaline in the diet. Belo (1990) found that serum insulin was lower in sheep in environments of 35°C and 55% RH compared to sheep in environments of 20°C and 64% RH. However, Achmadi *et al.* (1993) found no significant differences in serum insulin in sheep between environmental temperatures of 30°C and 20°C. It has been well documented that cold exposure reduces serum insulin levels in sheep (Sasaki & Takahashi, 1980; Sasaki *et al.*, 1982; Thompson *et al.*, 1982). However, the effects of heat exposure on serum insulin in sheep are poorly documented in comparison, which leads to uncertainty in the interpretation of the results in the present trial.

Insulin has important roles in partitioning of nutrients between the mammary and other tissues. In this role, insulin works in association with growth hormone in an antagonistic relationship (Vernon, 1982). High growth hormone concentrations relative to insulin concentrations favour milk production (Vernon, 1980; Vernon *et al.*, 1981). Mellor *et al.*

(1987) suggested that a high growth hormone to insulin ratio can also promote udder development.

Growth hormone levels were not measured in the present trials so possible involvement in insulin secretion differences between treatment groups cannot be determined. However, levels of growth hormone have been observed to be increased in cattle grazing E+ tall fescue pastures (Thompson *et al.,* 1987), and, conversely, Rice *et al.* (1995) found that growth hormone concentrations were decreased in cattle grazing E+ tall fescue pastures. Recently Browning *et al.* (1997) found that the synthetic ergopeptine alkaloid, ergotamine, intravenously administered, increased serum growth hormone levels in steers. It is, therefore, possible that ergovaline may affect serum insulin levels via direct affects on growth hormone secretion.

4.5. Mammogenesis and lactogenesis

Differences in mammogenesis (as measured by mammary girth) observed in the high temperature room in Trial 2 might be associated with significant disruptions of the major regulating hormones in the Ev+ group. Conversely, in Trial 1 and in the low temperature room in Trial 2, there did not appear to be any differences in mammogenesis. Lactogenesis was not possible in the Ev+ high temperature group as there had been no apparent mammogenesis. There was no significant difference in the onset of lactogenesis between the Ev+ and Ev- groups in Trial 1, however, lactose levels were significantly higher in the milk of the Ev- ewes. The difference in lactose levels may have been due to differences in insulin levels and hence partitioning of nutrients within the mammary gland. However, this cannot be verified, as serum insulin levels were not measured in Trial 1.

During gestation mammogenesis requires the action of oestrogens and progesterone followed by prolactin and/or placental lactogen, and glucocorticoids. At parturition, marked hormone changes effect a switch from colostrum production to milk secretion, crucial steps in this transition being a decrease in plasma progesterone concentration and increases in prolactin and glucocorticoid levels (Mellor *et al.,* 1987). Udder development is also affected by the ratio of growth hormone to insulin during pregnancy (Mellor *et al.,* 1987). It is likely that the disruption of one or many of these hormones by ergovaline and high ambient temperature is

responsible for the significant effects on mammogenesis observed in the Ev+ ewes in the high temperature room.

Prolactin has an important role in lactogenesis in the ewe (Peterson, 1992), although the importance of prolactin in mammogenesis in the ewe is in question due to the involvement of placental lactogen.

It is uncertain whether lower prolactin in the Ev+ ewes compared with the Ev- ewes in the high temperature room is responsible for the total suppression of mammary development in the Ev+ ewes. Despite the significant suppressive effects of ergovaline, prolactin levels in the Ev+ ewes in the high temperature room were significantly greater than those of both groups in the low temperature room and in Trial 1, where no impairment in mammary development was apparent. Therefore lower levels of prolactin *per se* could not have caused the lack of mammary development in these ewes.

Additionally, Hooley *et al.* (1978) and Schams *et al.* (1984) considered it possible that ovine placental lactogen may render prolactin unnecessary for mammogenesis. The fact that sheep do not exhibit agalactia whereas horses and rabbits, that do not have placental lactogen (Forsyth, 1986), do exhibit agalactia when prolactin levels are reduced by ergovaline, is evidence of this. Therefore the suppression of prolactin by ergovaline is unlikely alone to result in the total lack of mammary development observed in the Ev+ ewes in the high temperature room.

Progesterone appears to be the major factor inhibiting the onset of lactation during pregnancy in the sheep (Hartmann *et al.*, 1973). Withdrawal of progesterone in combination with a surge in cortisol are major influences in potentiating the lactogenic action of prolactin (Delouis *et al.*, 1980). Prior to parturition in sheep, the threshold below which the progesterone concentration of peripheral plasma must decrease before mammary blood flow can increase is about 10 ng/ml (Burd *et al.*, 1978) and the threshold for lactogenesis is about 1 ng/ml (Hartmann *et al.*, 1973). At parturition the serum progesterone levels had fallen below 10 ng/ml in all treatment groups. Therefore progesterone levels are not likely to have limited blood flow to the mammary glands. However, in the E+ group in the high temperature room, progesterone levels remained above the 1 ng/ml threshold, below which lactogenesis is

initiated, until 2 days *postpartum*, whereas, progesterone in the other groups had fallen below this level by parturition. This may have contributed to the lack of milk production in the Ev+ high temperature ewes. However, this does not explain the apparent lack of mammary development in these ewes. As with prolactin, the levels of serum progesterone *per se* were unlikely to have accounted for differences in mammary development between the Ev+ high temperature ewes and the other groups of ewes. This suggestion is supported by the fact that significant differences in serum progesterone between the Ev+ and Ev- groups in the low temperature room in Trial 2 were not associated with differences in mammogenesis and lactogenesis.

Although cortisol is also an important hormone in mammogenesis and in particular in lactogenesis, there were no significant differences between any of the treatment groups and cortisol is therefore, is unlikely to have caused any differences in either process.

The current study is limited in allowing interpretation of differences in mammary development in that, important hormones controlling mammogenesis such as growth hormone and oestradiol were not measured and the possible influences of thyroid hormones cannot be determined.

Results in this trial indicated that high ambient temperature was necessary to precipitate the degree of mammogenic suppression observed. The degree to which high ambient temperature affected mammary development indirectly by exacerbating the effects of ergovaline on important hormones or directly by its own effects on hormone levels is unclear. It is likely that a combination of indirect and direct effects was responsible. Given that there were no significant differences in mammary development between Ev- ewes in the high temperature room and Ev- ewes in the low temperature room it would appear that the indirect effects were of more importance. However, it has been found that sheep in high temperature environments often have smaller mammary glands and decreased milk yield that may be associated with redistribution of blood flow in the body (Berman, 1991). Assuming this is correct, the effects of ergovaline on vascular tissues may compound this blood flow problem.

It is highly unlikely that the severe effects of ergovaline on mammary development observed in the high temperature room in this trial would be exhibited in ewes grazing E+ pasture. Ewe

flocks in New Zealand generally start lambing in early spring when ambient temperatures are generally low. In addition, ergovaline levels in the pasture are also low at this time of the year and never reach levels similar to those used in the diets for the present trials. However, this research may have important implications for the mammary development of ewe lambs before puberty. A major phase of growth occurs in the parenchymal tissues of the mammary gland in female lambs before 20 weeks of age (Johnsson & Hart, 1985). By 20 weeks of age, spring-born lambs on endophyte-infected ryegrass pastures will have been grazing these pastures during the late spring and summer when both ambient temperature and ergovaline levels can be high. It is possible that this combination of higher ambient temperature and ergovaline in the diet may adversely affect mammary development in these female lambs. Administration of the ergot alkaloid bromocriptine has been shown to reduce parenchymal development, prolactin and insulin levels in female lambs compared with untreated lambs (Johnsson *et al.*, 1986). It is possible that ergovaline will have similar effects.

Similarly, dairy cow herds that are calved in the autumn for winter milk supply may be exposed to ambient temperatures and toxin levels during the autumn that are sufficient to affect mammary development in these herds.

4.6. Thermoregulation

Rectal temperatures were significantly higher in the Ev+ ewes than in the Ev- ewes in Trial 1 and in the high temperature room in Trial 2. Increased body temperature has been recorded previously in sheep fed diets containing ergovaline (Gadberry *et al.*, 1997), and is a common occurrence in sheep grazing endophyte-infected pastures during warm weather (Fletcher *et al.*, 1999).

The ergopeptine alkaloids have vasoconstrictive activity, thus reducing peripheral blood flow and hence the animals' ability to dissipate excess heat through the skin (Rhodes *et al.*, 1991). Vasoconstriction and bronchoconstriction would also complicate heat loss from the lungs (Oliver, 1997).

Additionally, prolactin may have an important role in thermoregulation in sheep (Salah *et al.*, 1995). Normally increases in ambient temperature are associated with large increases in the

secretion of prolactin in sheep (Hill & Alliston, 1981). This was clearly shown in Trial 2, where a large increase in serum prolactin was observed in the E- group of ewes in the high temperature room.

However, in animals fed ergovaline, the normal prolactin response to increased ambient temperature is impaired and consequently the ewes may not be able to regulate their body temperature adequately. Faichney & Barry (1986) found that suppression of prolactin secretion with bromocriptine impaired ewes' ability to maintain their body temperature under warm conditions (30°C).

The suppression of prolactin by ergovaline in the high temperature room was related to an increase in rectal temperature in the Ev+ ewes indicating an inability to maintain body temperature. Prolactin levels in the Ev+ ewes were inversely related ($P < 0.05$) to rectal temperature. This suggests that the ewes, in which prolactin was suppressed the most, were unable to regulate their temperature as well, and consequently suffered from higher body temperature.

However, differences in rectal temperature were observed between the Ev+ and Ev- ewes in Trial 1 where there was no significant difference in serum prolactin levels. This demonstrates that, although suppressed prolactin secretion may be an important factor in impaired thermoregulation in animals grazing endophyte-infected pastures, body temperature differences will still occur due to the other effects of endophyte toxins on vascular and pulmonary smooth muscle.

4.7. Maternal behaviour

The Ev+ ewes in the high and low temperature environments were reluctant to allow their lambs to suckle. This poor maternal behaviour was not observed in any of the Ev- ewes. Maternal behaviour is regulated by the hormonal changes that occur at the time of parturition. Oestrogens are thought to be essential (Siegel & Rosenblatt, 1975). Oestrogens and progesterone are believed to be important in regulating maternal behaviour in the ewes as they can be used to artificially induce maternal behaviour in nulliparous ewes (Le Neindre *et al.*, 1979). Progesterone appears to inhibit the appearance of maternal drive (Numan *et al.*, 1977)

It is possible that the disruptions in prolactin and in particular progesterone secretion in the Ev+ ewes will have had adverse effects on the establishment of normal maternal behaviour that follows parturition. It appears that maternal behaviour will only be affected if the hormonal regulation is upset in the first 4 to 8 hours after parturition during which time the dam/young bonds are formed. Changing the hormone profile in lactating ewes with bromocriptine after this period failed to alter normal maternal behaviour (Kann *et al.,* 1977).

Differences in maternal behaviour have not been examined between ewes grazing E+ and E- ryegrass pastures, although large differences are unlikely to be observed as toxin levels in the pasture at lambing are generally too low to affect the hormonal regulation of this behaviour. As with mammary development, maternal behaviour may be affected by ergovaline in autumn lambing ewes.

4.8. Faecal moisture

Faecal moisture increased rapidly when ergovaline was introduced into the diet. When ergovaline was removed the faecal moisture did not return to normal within the subsequent 6-day period, which suggests that the physiological mechanisms regulating water uptake by the intestine take time to recover from the adverse effects of ergovaline.
Serum prolactin levels were not measured in the ewes used for the faecal moisture trial, however, it is possible that the depression of prolactin by ergovaline precipitated the increase in faecal moisture. Prolactin has been found to have regulatory roles in water uptake by the mammalian intestine (Shennan, 1994), therefore a depression of prolactin will reduce effectiveness of this water uptake and hence increase the amount passed in the faeces.

These results indicate that ergovaline may be one of the major causes of increased faecal moisture in sheep grazing E+ ryegrass pastures, which promotes increased formation of dags in these animals. The possible effects of other endophyte toxins on faecal moisture are not clearly understood and should not be disregarded. However, sheep grazing endophyte/ryegrass associations that do not produce lolitrem B, but still produce ergovaline, have a similar prevalence and severity of dags to those grazing a wild-type association (Fletcher *et al.,* 1999). This also implicates ergovaline as a major factor responsible for the increase in faecal moisture associated with grazing E+ pastures.

4.9. Conclusions

The ergopeptine alkaloid ergovaline suppressed serum prolactin in sheep during the last 20 days of gestation under conditions of high ambient temperature. Ergovaline also suppressed progesterone levels during late pregnancy at both high and low ambient temperatures. Neither ergovaline nor ambient temperature affected serum cortisol levels.

Ewes that were exposed to both ergovaline and high ambient temperature during the last 20 days of pregnancy were agalactic, which appeared to be associated with disruptions in the endocrine systems regulating mammary development.

Ewes in New Zealand flocks usually lamb in early spring and are not likely to be exposed to the high ergovaline levels in the feed and the ambient temperature conditions in Trial 2. Therefore it is highly unlikely that the affects on mammary development observed in Trial 2 will be observed in ewes grazing E+ ryegrass pastures. However, this research may have implications for mammary development and lactation in ewes that lamb in autumn having undergone mammary development during the late spring and summer when pasture ergovaline levels and ambient temperatures can be high. Peterson *et al.* (1990) found that milk yields and lamb growth rates were lower in autumn born than spring born flocks grazing perennial ryegrass pastures, which was associated with lower circulating prolactin levels. It is possible that this may have been due to the effects of higher endophyte toxins in the pasture during autumn. Additionally, the hormone systems controlling mammogenesis and lactogenesis in the pregnant ewe also control mammary development in prepubertal ewe lambs. In spring born ewe lambs the mammary gland will be developing during the late spring and summer. Therefore, high toxin levels in the pasture and high ambient temperatures during this period may adversely affect the mammary development in these animals.

CHAPTER VIII

Feed intake and grazing behaviour of ewes and lambs grazing either endophyte-infected or endophyte-free perennial ryegrass

1. Introduction

Reduced liveweight gain is one of the most commonly observed maladies in animals grazing endophyte-infected pasture. It has been shown that sheep grazing E+ perennial ryegrass have lower average daily gains than sheep grazing E- ryegrass (Fletcher & Barrell, 1984). One of the consistent effects of endophyte observed in the grazing trials described in Chapters III and IV of this study, was the lower liveweight of the ewe flocks grazing endophyte-infected pastures than those grazing endophyte-free pastures. This difference occurred despite the same pasture allowance being offered to each group.

Ewe condition can be an important determinant of reproductive performance. Therefore, reduced ewe condition due to grazing endophyte-infected pasture is likely to indirectly affect ewe fertility.

The most likely major cause of this reduction in liveweight is reduced voluntary feed intake. Fletcher & Barrell (1984) suggested that reduced growth rate in sheep grazing E+ ryegrass may have been due to reduced herbage intakes. Since then it has been observed that cattle, horses and sheep will consume less E+ tall fescue forage than E- forage. (Jackson *et al.,* 1988; Redmond *et al.,* 1991; Chestnut *et al.,* 1992). There is a need for similar studies to be conducted examining feed intake in animals grazing E+ perennial ryegrass.

The reasons for lower intake in animals grazing E+ pasture are unclear, but may be due to a change in grazing behaviour induced by the presence of toxins. A reluctance of sheep to graze the pseudostem horizon of E+ ryegrass pasture was observed by Edwards *et al.* (1993). Higher toxin levels are found in the pseudostem component of E+ pasture than in the leaf-blade component (Keogh *et al.,* 1996), and it is likely that this could be the cause of a reluctance of sheep to graze into this horizon.

There have been no reports that link this difference in grazing behaviour and voluntary feed intake in animals grazing E+ pasture. The objectives of this study were to: 1) Determine the effects of endophyte on voluntary feed intake and grazing behaviour in ewes and suckling lambs. 2) Relate these effects to the ewe and lamb liveweight differences observed in the grazing trials.

2. Materials and methods

2.1. Animals

Two groups of ewes (n=35) with lambs at foot (17 twin-rearing and 18 single-rearing), that had been grazing either endophyte-infected or endophyte-free perennial ryegrass for three years, were orally dosed with intra-ruminal chromium sesquioxide controlled release capsules (Captec (NZ) ltd.). Twelve single and eight twin lambs from each group, heavier than 25 kg liveweight, were also dosed with capsules.

All ewes and lambs were weighed prior to the start and at the completion of the grazing trial.

2.2. Grazing management

The groups of ewes and lambs were break-fed behind an electric fence on their respective treatments with the same average pre-grazing herbage mass, and same grazing area (0.2 ha) (Plate 8.1.). The daily pasture allowance was approximately 2 kgDM per ewe + lamb(s). The groups grazed each break for six days before being offered a fresh break. The groups grazed 5 six-day breaks during the trial period.

The treatment pastures had the same pre-grazing botanical composition (see Table 8.7. and Plates 8.2. and 8.3.).

The groups were given *ad libitum* access to water throughout the trial.

Plate 8.1. Ewes and lambs grazing treatment pastures

Plate 8.2. Pre-grazing E- pasture

Plate 8.3. Pre-grazing E+ pasture

2.3. Use of controlled release chromium oxide capsules

The use of chromium tracer in capsule form was considered the best method available for the determination of feed intake under the grazing management employed in this trial. The use of capsules allowed a reduction in the labour requirement, animal handling and errors associated with daily chromium dosing. It also allowed application of the technology to lambs (>25 kg liveweight).

With the capsules a uniform rate of release is generally achieved 2 to 3 days after administration. However, steady state levels of Cr_2O_3 in the faeces, at maintenance levels of intake, are usually not achieved until day 7 or 8 post-administration in sheep (Parker *et al.*, 1990).

There is usually an 18-day period of linear release of chromium from the capsule. Actual chromium release can be determined by recovery of the capsule from animals after slaughter at appropriate times but this was not practical for the present trial. Therefore an alternative method was used to estimate chromium release described by Ellis *et al.* (1988). This was achieved by determining the time of chromium disappearance from the faeces i.e. 'end point' determination. There is no evidence to suggest that the chromium release rate would differ between ewes and lambs, as even interspecies differences are small.

The effects of level of feed intake and feed type on the rate of chromium release are usually small, however, changes in chromium release can occur when feeding levels are changed (Parker *et al.*, 1989). This may have implications in the rotational grazing method employed in the present trial. However this potential problem was minimised by the relatively lax grazing pressure on each break. Additionally, the chromium data collected appeared to follow similar patterns within each break in both treatment groups indicating some consistency.

2.4. Faecal sampling

After an 8-day equilibration period to establish a constant chromium oxide release from the capsules, daily faecal samples were collected from the rectum between days 8 and 30 from capsule administration from all ewes and lambs that had received a chromium capsule. Faecal sampling continued 5 days past the chromium release period stated by the manufacturer (25

days from capsule administration) to determine the endpoint of chromium release. Mean daily chromium oxide release from the capsule was estimated to be 171 mg of active chromium oxide per day.

The faecal samples were freeze-dried and then ground. Daily (d8 to d30) faecal samples from 2 twin-rearing and 2 single-rearing ewes, and 2 twin lambs and 2 single lambs, in each group were analysed for chromium to determine the period of constant chromium oxide release (d8 to d26). There were only two complete grazing breaks during the period of constant chromium release (d13 to d18, and d19 to d 24). Analysis results of the daily sampling from the 4 ewes and 4 lambs in each group showed that a consistent faecal chromium pattern existed within each of the two grazing breaks in the constant release period (i.e. faecal chromium levels increased between the start and end of each break). Given that this similar pattern was present in each break, the large number of samples gathered (>2000), and the relatively high cost of chromium analysis, it was decided that faecal samples could be analysed for each animal during only one grazing break within the constant chromium release period (d13 to d18).

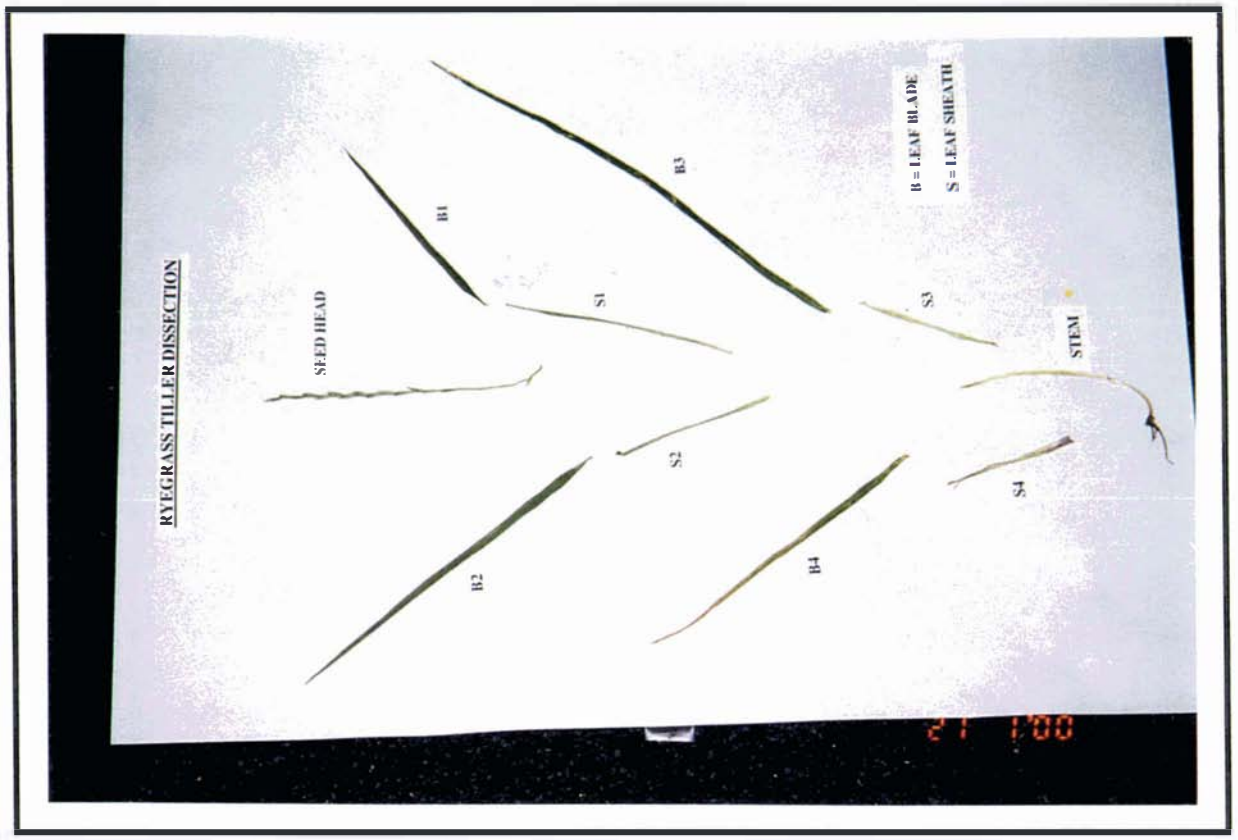
2.5. Herbage sampling

Three pre- and three postgrazing quadrat (20 cm x 50 cm) cuts were taken from each of the 5 breaks on the E+ and E- pastures. Each herbage sample was weighed and a 100g subsample was oven dried to determine dry matter percentage, and the average pre- and postgrazing pasture cover (kgDM/ha). A further 100g subsample was dissected into ryegrass leaf-blade, leaf-sheath, clover, other grass species, and dead material, then freeze-dried and weighed to determine the botanical composition of the pastures before and after grazing. The ryegrass leaf-blade and leaf-sheath from the second subsample were ground, pooled across breaks, and analysed for lolitrem B and ergovaline levels.

Fifty vegetative ryegrass tillers were collected randomly from the E+ and E- pastures prior to grazing and a further fifty tillers were collected after each break had been grazed for 6 days. Each tiller was dissected into leaf blade and sheath of different age, seed head and stem (see Plate 8.4.). The length of each pre- and post-grazing component was measured. Each of the dissected components was pooled within pasture type, freeze-dried, and weighed to determine the total pre- and postgrazing dry weight of each component and the amount of each

component removed by the sheep from the 50 tiller sample. After weighing the pre-grazing herbage components were pooled across grazing breaks and analysed for lolitrem B and ergovaline.

Plate 8.4. Ryegrass tiller dissection



2.6. Herbage ergovaline and lolitrem B analyses

Freeze-dried and ground ryegrass samples were measured for ergovaline and lolitrem B levels by HPLC as described by Barker *et al.* (1993) at the Plant Biochemistry Laboratory, AgResearch Grasslands, Palmerston North (see Chapter III, section 2.5. for further details).

2.7. Chromium analysis

Faecal chromium oxide was determined by plasma emission spectrometry at the AgReserach ICP Facility, Grasslands Research Centre, Palmerston North.

2.8. Statistical analyses

All statistical analyses were carried out using Graph-pad Prism™ 3.0 computer software: 10855 Sorrento Valley Rd #203, San Diego, CA 92121 USA.

Faecal chromium levels in the ewe and lambs were analysed by generalised linear model (GLM) to determine the source of variation due to treatment (endophyte), rearing status (single vs twin (rearing)) and time (day of grazing each break).

Ewe and lamb faecal chromium levels were adjusted to a common liveweight using the equation: $FC_2 = FC_1 \text{ X } LW_I / LW_M$
where FC_2 = the adjusted faecal chromium level, FC_1 = the unadjusted faecal chromium level, LW_I = the individual ewe/lamb liveweight and LW_M = the mean liveweight of all ewes/lambs.
Ewe and lamb liveweight and liveweight change were analysed by ANOVA.

Multiple regression analysis was used to determine the variance in faecal chromium level of ewes and lambs due to liveweight and liveweight change.
Pasture growth rate and nutritional parameters were analysed by ANOVA.
Ryegrass component measurements were analysed by two-way ANOVA to determine source of variance due to endophyte status, component type and component X endophyte interaction.

Pasture composition data were analysed by chi-square test.

3. Results

3.1. Faecal chromium oxide levels

3.1.1. Ewes

Faecal chromium oxide levels for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture on a six day break are shown in Table 8.1.

Faecal chromium levels in the twin-rearing ewes were not significantly different between the E+ and E- group on any day of grazing. Faecal chromium levels in the twin-rearing ewes were significantly ($P<0.01$) affected by time (day of grazing on each break), with levels increasing with time. There was no significant interaction between time and treatment (endophyte status of the pasture) in the twin-rearing ewes.

Faecal chromium levels in the single-rearing ewes were significantly ($P<0.01$) higher in the E+ group than the E- group on days 2, 5 and 6 of grazing. Faecal chromium levels in the single-rearing ewes were significantly ($P<0.01$) affected by time (day of grazing on each break), with levels increasing with time. There was no significant interaction between time and treatment.

Table 8.1. Mean (±SEM) faecal chromium oxide levels (μg/g) for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.

DAY OF BREAK	TWIN-REARING		SINGLE-REARING	
	E+	E-	E+	E-
1	262 ± 29	265 ± 21	292 ± 23	297 ± 21
2	260 ± 30^{a}	270 ± 23^{a}	370 ± 51^{b}	257 ± 32^{a}
3	296 ± 30	302 ± 26	344 ± 45	292 ± 30
4	351 ± 21	357 ± 45	398 ± 38	368 ± 33
5	382 ± 24^{a}	403 ± 36^{a}	418 ± 36^{a}	323 ± 20^{b}
6	398 ± 23^{a}	351 ± 38^{a}	487 ± 31^{b}	375 ± 17^{a}

NB: Means with different superscript letters within a row differ significantly ($P<0.01$).

3.1.2. Lambs

Faecal chromium oxide levels for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture on a six day break are shown in Table 8.2.

Faecal chromium levels in the twin lambs were significantly ($P<0.01$) higher in the E+ group than in the E- group on days 2, 4, 5 and 6 of grazing. Faecal chromium levels in the twin lambs were significantly ($P<0.05$) affected by time (day of grazing on each break), with levels increasing with time. There was no significant interaction between time and treatment.

Faecal chromium levels in the single lambs were significantly ($P<0.01$) higher in the E+ group than in the E- group on days 4 and 6 of grazing. Faecal chromium levels in the single lambs were significantly ($P<0.05$) affected by time (day of grazing on each break), with levels increasing with time. There was no significant interaction between time and treatment.

Table 8.2. Mean (±SEM) faecal chromium oxide levels (μg/g) for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.

DAY OF	TWIN LAMBS		SINGLE LAMBS	
BREAK	E+	E-	E+	E-
1	650 ± 58	627 ± 69	563 ± 82	481 ± 59
2	730 ± 60^{a}	449 ± 80^{b}	565 ± 86^{ab}	553 ± 68^{ab}
3	719 ± 64	605 ± 52	708 ± 63	578 ± 69
4	761 ± 59^{ab}	583 ± 60^{c}	784 ± 52^{a}	634 ± 54^{bc}
5	774 ± 48^{a}	533 ± 60^{b}	688 ± 82^{ab}	585 ± 69^{b}
6	836 ± 40^{a}	678 ± 76^{bc}	803 ± 73^{ac}	527 ± 89^{b}

NB: Means with different superscript letters within a row differ significantly ($P<0.01$).

3.2. Ewe liveweight and liveweight change

The pre- and post-trial ewe liveweight and daily liveweight change of twin and single-rearing ewes in the E+ and E- groups is shown in Table 8.3.

Single-rearing ewes in the E- group were significantly ($P<0.01$) heavier than all other groups of ewe before the trial period. There were no significant differences in pre-trial ewe liveweight between any other group of ewes. After the trial period, the E- single-rearing ewes remained significantly ($P<0.01$) heavier than all other groups of ewes. The twin-rearing ewes in the E- group were significantly ($P<0.01$) heavier than the single rearing ewes in the E+ group post-trial but were not significantly different from the E+ twin-rearing ewes.

Single and twin-rearing ewes grazing E+ pasture lost weight on average during the trial period whereas the twin and single-rearing ewes grazing the E- pasture put weight on during the trial period.

Table 8.3. Mean (±SEM) pre- (PRE-LW) and post-trial (POST-LW) liveweight (kg) and liveweight change (LWΔ) (g/d) of twin and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.

	TWIN-REARING		**SINGLE –REARING**	
	E+	**E-**	**E+**	**E-**
PRE-LW	60.7 ± 2.4^{a}	59.5 ± 2.5^{a}	57.5 ± 2.3^{a}	65.5 ± 1.5^{b}
POST-LW	58.4 ± 2.0^{ab}	62.1 ± 1.8^{a}	54.9 ± 2.2^{b}	65.7 ± 1.2^{c}
LWΔ	-67 ± 16^{a}	75 ± 30^{b}	-73 ± 15^{a}	6 ± 18^{c}

NB: Means with different superscript letters within a row differ significantly ($P<0.01$).

3.3. Lamb liveweight and liveweight change

The pre- and post-trial liveweight and daily liveweight change of twin and single-lambs in the E+ and E- groups is shown in Table 8.4.

Pre-trial liveweight of the E- single lambs was significantly (P<0.01) heavier than all other groups of lambs. There were no significant differences in pre-trial liveweight between the E+ single lambs and the E- twin lambs, however these two groups of lambs were significantly (P<0.01) heavier than the E+ twin lambs. After the trial period the liveweight of the E- single lambs remained significantly (P<0.01) heavier than all other groups of lambs and E+ single lambs were significantly (P<0.01) heavier than E+ twin lambs. The difference in the liveweight of E+ and E- twin lambs was not significantly different after the trial period, however, the E- lambs remained heavier than the E+ lambs.

There were no significant differences in daily growth rate between any group of lambs throughout the trial period.

Table 8.4. Mean (±SEM) pre- (PRE-LW) and post-trial (POST-LW) liveweight (kg) and liveweight change (LWΔ) (g/d) of twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.

	TWIN LAMBS		SINGLE LAMBS	
	E+	**E-**	**E+**	**E-**
PRE-LW	23.7 ± 0.6^{a}	27.3 ± 0.6^{b}	27.6 ± 1.1^{b}	31.7 ± 1.3^{c}
POST-LW	30.8 ± 1.0^{a}	33.5 ± 1.4^{ab}	34.2 ± 1.3^{b}	38.3 ± 1.6^{c}
LWΔ	202 ± 14	177 ± 13	188 ± 11	188 ± 15

NB: Means with different superscript letters within a row differ significantly (P<0.01).

3.4. Relationship between ewe liveweight, liveweight change and faecal chromium levels

Faecal chromium oxide levels, adjusted for pre-trial ewe liveweight, for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture are presented in Table 8.5.

There were no significant differences in faecal chromium levels that were adjusted for ewe liveweight between any group of ewes on days 1 to 5 of grazing the break. Faecal chromium

levels were significantly greater in the E+ single-rearing ewes than the twin rearing ewes in the E+ and E- group on day 6 of grazing, but were not significantly different from the single rearing E- ewes.

Multiple regression analysis of pre-trial ewe liveweight and liveweight change on faecal chromium found that 14% (significant, $P<0.01$) of the total variance in faecal chromium was due to liveweight and 7% (significant, $P<0.01$) was due to liveweight change during the trial period.

Multiple regression equation:

$FC = 730 - 496LW\Delta - 5.45LW$

where FC = faecal chromium, LWΔ = liveweight change and LW = pre-trial liveweight.

Table 8.5. Mean (±SEM) faecal chromium oxide levels (µg/g), adjusted for pre-trial ewe liveweight, for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.

DAY OF BREAK	TWIN-REARING		SINGLE-REARING	
	E+	E-	E+	E-
1	263 ± 24	269 ± 20	274 ± 28	302 ± 21
2	266 ± 35	274 ± 36	305 ± 28	258 ± 32
3	285 ± 30	299 ± 26	310 ± 20	301 ± 30
4	338 ± 19	361 ± 62	383 ± 31	394 ± 35
5	374 ± 20	402 ± 34	393 ± 32	352 ± 25
6	389 ± 26[a]	348 ± 53[a]	455 ± 29[b]	399 ± 19[ab]

NB: Means with different superscript letters within a row differ significantly ($P<0.01$).

3.5. Relationship between lamb growth rate, liveweight and faecal chromium levels

Faecal chromium oxide levels, adjusted for lamb pre-trial liveweight, for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture are presented in Table 8.6.

There were no significant differences in faecal chromium levels that were adjusted for lamb liveweight between any group of lambs on days 1 to 3 of grazing the break. Faecal chromium

levels were significantly (P<0.01) lower in the E- twin lambs than in all other groups of lambs on day 4 and higher in E+ twin lambs than in all other groups of lambs on day 5. Faecal chromium levels were significantly (P<0.01) lower in the E- twin and single lambs than in the E+ twin and single lambs on day 6.

Multiple regression analysis of pre-trial lamb liveweight and liveweight change on faecal chromium, across all treatment groups and rearing status, found that 7% (significant, P<0.05) of the total variance in faecal chromium was due to liveweight, however, liveweight change during the trial period was not a significant (<1%) source of variation.

Multiple regression equation:

$FC = 1004 + 1167LW\Delta - 14.5LW$

where FC = faecal chromium, LWΔ = liveweight change and LW = pre-trial liveweight.

Table 8.6. Mean (±SEM) faecal chromium oxide levels (μg/g), adjusted for lamb pre-trial liveweight, for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.

DAY OF BREAK	TWIN LAMBS		SINGLE LAMBS	
	E+	E-	E+	E-
1	598 ± 69	572 ± 74	542 ± 74	538 ± 63
2	661 ± 60	450 ± 120	543 ± 79	612 ± 76
3	650 ± 62	574 ± 58	683 ± 64	632 ± 73
4	707 ± 46^{a}	522 ± 22^{b}	756 ± 54^{a}	711 ± 50^{a}
5	713 ± 58^{a}	528 ± 108^{b}	663 ± 80^{ab}	642 ± 63^{ab}
6	736 ± 44^{a}	599 ± 72^{b}	795 ± 76^{a}	591 ± 105^{b}

NB: Means with different superscript letters within a row differ significantly (P<0.01).

3.6. Pasture growth rate

There were no significant differences in daily pasture growth rate between the E+ and E- pastures during the trial period. The daily pasture growth rates during the trial period were 104 ± 20 kgDM/ha/d and 97 ± 15 kgDM/ha/d for the E+ and E- pastures respectively.

3.7. Pasture nutritional analysis

There were no significant differences in any of the nutritional parameters measured between the E+ and E- pasture.

For mean (±SEM) protein, lipid, ash, acid detergent fibre, neutral detergent fibre, soluble carbohydrate, organic matter digestibility, and metabolisable energy in E+ and E- ryegrass pasture during the trial period refer to data for spring presented in Table 3.9. in Chapter III.

3.8. Ergovaline and lolitrem B levels in the pasture and distribution within the ryegrass plant

Mean (±SEM) ergovaline levels in the E+ pasture were 0.3 ± 0.2 ppm and 0.8 ± 0.3 ppm for the ryegrass leaf blade and leaf sheath respectively. No ergovaline was detected in the E- pasture.

Mean (±SEM) lolitrem B levels in the E+ pasture were 0.4 ± 0.1 ppm and 2.0 ± 0.4 ppm for the ryegrass leaf blade and leaf sheath respectively. No lolitrem B was detected in the E- pasture.

Ergovaline and lolitrem B concentrations in the leaf blades and leaf sheaths of different ages and seed head and stem components in the E+ ryegrass plant are shown in Table 8.7.

Toxin levels in the leaf components of different age presented in Table 8.7. are lower than those measured in the bulk leaf blade and sheath samples. This may have been due to the fact that the bulked samples contained grazed plants. Toxin levels increase from the blade tip to the base of the blade where it joins the sheath (Keogh *et al.,* 1996). Grazed samples will therefore contain a higher proportion of the basal portion of leaf blade, which will increase the average toxin levels for that component.

Table 8.7. Pre-grazing ergovaline and lolitrem B concentration (ppm) in leaf blades and leaf sheaths of different age, seed head and stem components of the E+ ryegrass plant

COMPONENT	ERGOVALINE	LOLITREM B
SEED HEAD	1.20	0.41
LEAF BLADE (LB)		
LB-1	0.20	0.13
LB-2	0	0.12
LB-3	0	0.21
LB-4	0	0.35
LEAF SHEATH (LS)		
LS-1	0.20	0.31
LS-2	0.20	0.47
LS-3	0	1.25
LS-4	0.20	2.73
STEM	0.70	0.68

3.9. Pre- and post-grazing tiller dimensions

Pre- and postgrazing lengths of leaf blades (B1-4) and sheaths (S1-4) components of different age on the E+ and E- ryegrass plants are shown in Figure 8.2.

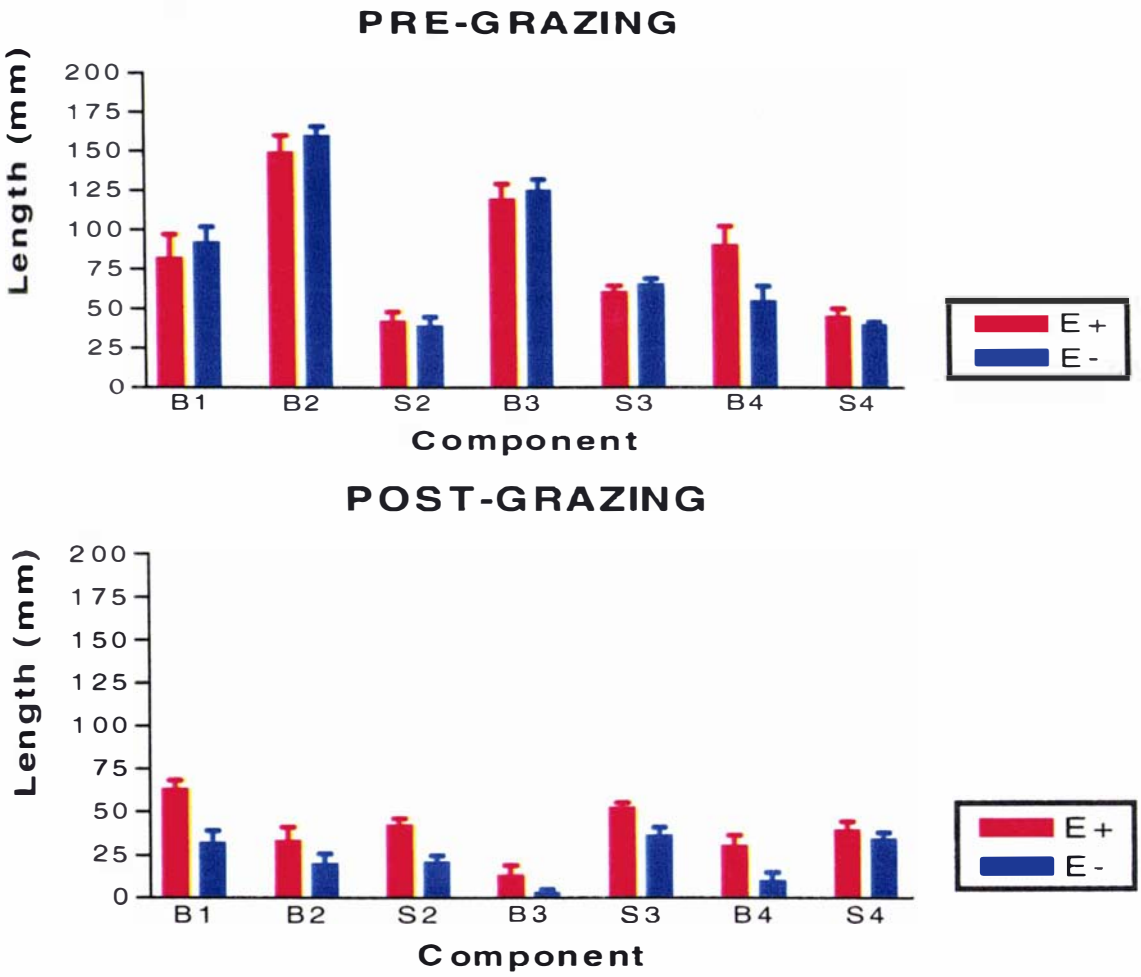
There were no significant differences in the pre-grazing length of any of the ryegrass components measured between the E+ and E- pastures. There were significant differences ($P<0.0001$) in the length of various components within the E+ and E- ryegrass tillers. The second youngest leaf blade (B2) was the longest component followed in order of decreasing length by B3, B1, B4 , S3, S2 and S4 (B1 and B4, and S2 and S4 were not significantly different in length). The B3, B4 and S4 components had been shortened by the previous grazing.

All postgrazing ryegrass components were significantly ($P<0.0001$) shorter in the E- plant than in the E+ plant. There were significant differences ($P<0.0001$) in the length of various components within the E+ and E- ryegrass plant. The youngest leaf blade (B1) was the

longest blade component followed in order of decreasing length by B2, B4 and B3 (B2 and B4 were not significantly different in length). There were no significant differences in the length of leaf sheath components within the E+ and E- ryegrass plant.

There was no significant pre- or post-grazing interaction between endophyte status and component type.

Figure 8.2. Mean (±SEM) pre- and post-grazing lengths (mm) of leaf blades (B1-4) and sheaths (S1-4) components of different age in the E+ and E- ryegrass plants.



Mean (±SEM) pre- and post-grazing weights (pooled from 20 tillers) of leaf blade (B1-4) and sheath (S1-4) components of different age from E+ and E- ryegrass tillers are shown in Table 8.8.

There were no significant differences in the pre-grazing weights of any of the ryegrass tiller components between the E+ and E- tillers. There were significant ($P<0.01$) differences in the

weight of components within a grass type with older components representing more of the total tiller weight than younger components. There was no significant grass type by component interaction.

Postgrazing component weights of the E- tillers were significantly ($P<0.001$) lower than the E+ tillers. Leaf sheath components made up significantly ($P<0.001$) more of the total tiller weight than leaf blade components after grazing. There was a significant ($P<0.01$) interaction between component and grass type postgrazing.

There was no significant difference in the pre-grazing leaf blade:sheath ratio between the E+ and E- tillers. The E+ tillers had a significantly ($P<0.001$) higher postgrazing blade to sheath ratio than the E- tillers.

Table 8.8. Mean (±SEM) pre- and post-grazing weights (µg dry-weight) (pooled from 20 tillers) of leaf blade (B1-4) and sheath (S1-4) components of different age, total blade (TB) and total sheath (TS) from 20 E+ and E- ryegrass tillers.

	PRE-GRAZING				POST-GRAZING			
	E+		E-		E+		E-	
COMP.	WT	%TOT	WT	%TOT	WT	%TOT	WT	%TOT
B1	93±17	8	69±10	5	83±6	17	28±6	10
B2	314±31	26	393±31	26	54±12	12	32±9	12
B3	229±41	19	298±30	20	17±7	4	7±6	3
B4	133±17	10	138±22	9	48±9	10	10±5	4
TB	769 ± 106	63	898 ± 93	60	202 ± 34	43	77 ± 26	29
S2	139±20	11	163±25	11	69±7	15	31±5	12
S3	211±25	17	316±34	21	118±7	25	70±10	27
S4	105±11	9	125±10	8	79±8	17	82±10	31
TS	455 ± 56	37	604 ± 69	40	266 ± 22	57	183 ± 25	71
TOTAL	1224 ± 165	100	1502 ± 162	100	468 ± 56	100	260 ± 46	100

3.10. Effects of grazing on pasture botanical composition

Pasture component dry-weight (kgDM/ha) and percentage of the pasture mass represented by each component before and after grazing, and the amount of each component removed (kgDM/ha) and its percentage of the total herbage removed during grazing is shown in Table 8.9.

There were no significant differences in the pre-grazing botanical composition between the E+ and E- pasture. After grazing perennial ryegrass represented a significantly greater proportion of the total herbage present in the E+ pasture than it did in the E- pasture (50% and 37% for the E+ and E- pastures respectively).

There were no significant differences in pre-grazing herbage mass between the E+ and E- pastures (2197 $\pm$ 437 kgDM/ha and 1918 $\pm$ 114 kgDM/ha for the E+ and E- pastures respectively). Significantly ($P<0.001$) less herbage was removed during the grazing period from the E+ pasture than from the E- pasture. This resulted in a significantly greater residual herbage mass on the E+ pasture than on the E- pasture (1126 $\pm$ 173 kgDM/ha and 647 $\pm$ 150 kgDM/ha for the E+ and E- pastures respectively).

A significantly ($P<0.001$) lower proportion of the pre-grazing ryegrass herbage was removed during grazing on the E+ pasture than on the E- pasture (46% and 79% respectively).

The ryegrass leaf blade portion of the total herbage removed from each pasture during grazing was not significantly different between the E+ and E- pastures. However, ryegrass leaf sheath was a significantly ($P<0.001$) lower proportion of the total herbage removed from the E+ pasture than it was from the E- pasture (5% and 22% respectively). Herbage components of the pasture other than perennial ryegrass made up a significantly ($P<0.001$) greater proportion of the total herbage removed from the E+ pasture than from the E- pasture (54% and 29% respectively).

Table 8.9. Dry-weight (kgDM/ha) and percentage of the pasture mass represented by ryegrass leaf blade (B1-4), sheath (S2-4), total blade (TB), total sheath (TS), total ryegrass (TR) other grass species (OG), clover (CL), dead material (D), and total non-ryegrass (TO), before and after grazing, and the amount of each component removed (kgDM/ha) and its percentage of the total herbage (T) removed during grazing.

	PRE-GRAZING				POST-GRAZING				REMOVED			
	E+		E-		E+		E-		E+		E-	
	WT	%	WT	%	WT	%	WT	%	WT Δ	%	WT Δ	%
LB1	88	4	58	3	90	8	26	4	-2	N/A	32	3
LB2	263	12	288	15	68	6	32	5	195	18	256	20
LB3	198	9	230	12	23	2	8	1	175	16	222	17
LB4	131	6	115	6	56	5	13	2	76	7	102	9
TB	**680**	**31**	**691**	**36**	**237**	**21**	**79**	**12**	**446**	**41**	**612**	**49**
LS2	110	5	115	6	90	8	26	4	20	2	89	7
LS3	176	8	230	12	146	13	64	10	30	3	166	13
LS4	88	4	96	5	90	8	71	11	-2	N/A	25	2
TS	**374**	**17**	**441**	**23**	**326**	**29**	**161**	**25**	**50**	**5**	**280**	**22**
TR	**1054**	**48**	**1132**	**59**	**563**	**50**	**239**	**37**	**491**	**46**	**893**	**79**
OG	769	35	633	33	338	30	277	43	431	40	354	28
CL	176	8	38	2	79	87	32	5	97	9	6	<1
D	198	9	115	6	146	13	97	15	52	5	18	1
TO	**1143**	**52**	**786**	**41**	**563**	**50**	**406**	**63**	**580**	**54**	**378**	**29**
T	**2197**	**100**	**1918**	**100**	**1126**	**100**	**647**	**100**	**1071**	**100**	**1271**	**100**

4. Discussion and conclusions

4.1. Faecal chromium and feed intake

In the two years prior to this trial, a significant liveweight difference had developed between ewes grazing E+ ryegrass pasture and those grazing the E- ryegrass pasture despite the amount of feed offered and grazing management being identical for both groups (see Chapter III, section 4.2.).

Results in the current trial indicate that reduced liveweight and liveweight gain in ewes grazing the E+ ryegrass pasture was due to a reduction in voluntary feed intake, as measured by chromium sesquioxide tracer. Multiple regression analysis (section 3.4.) of faecal chromium levels on ewe pre-trial liveweight and liveweight change during the trial period showed that these parameters were a significant source of variation in faecal chromium with pre-trial liveweight being the greatest contributing factor. Previous reports have suggested that E+ forage may have lower digestibility than E- forage (Aldrich *et al.*, 1993a; 1993b) which could be partially responsible for lower weight gains in animals grazing E+ pasture. However, nutritional analysis of the pastures used in the current trial failed to find a significant difference between the E+ and E- pasture in any of the nutritional parameters measured.

The relationship between ewe liveweight and feed intake was evident in the single-rearing ewes where a significantly greater faecal chromium level in the E+ ewes was associated with significantly lower liveweight. Alternatively, a lack of significant difference in the liveweight of twin-rearing ewes between the E+ and E- group was associated with a lack of significant difference in faecal chromium level. Further evidence of the relationship between feed intake and ewe liveweight was the reduction in the differences in faecal chromium levels between groups when adjusted to a common liveweight. However, even after faecal chromium levels were adjusted to a common liveweight, there was still a significant difference between the E+ and E- single-rearing ewes on day 6 of the break. This difference could reflect the greater weight loss by the E+ single-rearing ewes during the grazing period.

Despite similar pre-trial liveweight and what appeared to be similar feed intake during the trial period, the E+ twin-rearing ewes were losing weight during the trial period, whereas the

E- twin-rearing ewes were gaining weight. This may have been due to differences between the E+ and E- twin-rearing ewes in partitioning nutrients for maintenance, growth and lactation. Milk production data collected in 1997 and 1998 (Chapter III, section 3.1.5.), and serum prolactin levels measured during lactation in 1998 (Chapter III, section 3.1.2.) from the ewes used in this trial indicate that differences in milk production existed between the E+ and E- ewes. Although milk production during early lactation (weeks 1 to 5 from parturition) was lower by the E+ twin-rearing ewes than by the E- twin-rearing ewes, it is possible that milk production by the E+ twin-rearing ewes may not have fallen as quickly in late lactation as it did in the E- twin-rearing ewes. Therefore the E+ twin-rearing ewes may have still been producing significant amounts of milk during the feed intake trial whereas the E- twin-rearing ewes would have been producing comparatively very little or no milk at this time. As a consequence more nutrients would have been required for milk production by the E+ twin-rearing ewes at the expense of growth and maintenance of liveweight.

Feed intake, as measured by chromium tracer, was significantly lower in the E+ lambs than in E- lambs and this was evident in both twin and single lambs. Twin and single lambs in the E+ group were significantly lighter than their E- counterparts which was due to differences in growth rate during early lactation in response to lower milk production in the E+ ewes. As pasture became the most significant component of the lambs' diet the differences in growth rate between the groups diminished and were not significant during this trial. As with the ewes, multiple regression analysis (section 3.5.) of faecal chromium on lamb liveweight and liveweight change showed that liveweight was a significant source of variation in faecal chromium, however, liveweight change in the lambs was not a significant source of variation. When faecal chromium levels were adjusted to a common liveweight there were no significant differences between the groups of lambs.

Both ewe and lamb intakes in both treatment groups decreased significantly as the number of days grazing each break increased. This is consistent with other reports that show a reduction in the herbage intake of ewes and lambs as green herbage mass decreases (Gibb & Treacher, 1978; Jamieson & Hodgson, 1979). Jamieson & Hodgson (1979) found that as herbage mass decreased from 3000 to 1000 kgDM/ha, biting rate and grazing time increased, but insufficiently to offset the rapid fall in bite size. Edwards *et al.* (1993) found that grazing time was similar between ewes grazing E+ and E- pasture despite differences in the rate of herbage removal, which may indicate that bite size was more restricted in the E+ ewes.

Although there was no significant interaction between day of grazing and treatment in any group of ewes or lambs, the single bearing ewes and both twin and single lambs in the E- group were able to maintain their feed intake at a greater level than their E+ counterparts as the number of days grazing each break increased.

4.2. Grazing behaviour

The reluctance of sheep to graze the pseudostem horizon of E+ perennial ryegrass pasture has been reported previously (Edwards *et al.*,1993). This study concluded that the reduction in grazing preference for E+ pasture, shown by a slower rate of canopy height decline, is probably highly correlated with pasture intake and animal productivity.

Postgrazing residual herbage measurements in the current trial are consistent with the findings of Edwards, with the postgrazing herbage mass being significantly higher on the E+ pasture. These differences in postgrazing herbage mass were also observed during the grazing trial described in Chapter III, section 3.2.5.

Higher postgrazing herbage mass on E+ pasture appears to be related to a lower acceptability of the E+ ryegrass plant than the E- ryegrass plant to the grazing animal. Tiller dimension measurements of E+ and E- ryegrass plants showed that the grazing sheep removed significantly less of the leaf blade and sheath components of the E+ plant compared to the E- plant. More leaf-blade was removed than leaf-sheath on both E+ and E- plants, with more of the longer second and third oldest leaf-blades being removed than all other components. This is consistent with a previous study that showed that longer plant components and green leaf blade were more likely to be grazed, which is aided by their accessibility near the top of the canopy (Chapman & Clark, 1984).

Pre- and postgrazing botanical dissection (Table 8.9.) of the E+ and E- pastures showed that more of all herbage components present in the pasture were removed by sheep grazing the E- pasture. Sheep grazing the E+ pasture grazed the ryegrass plant population less intensively than sheep grazing E- pasture and this was particularly evident for the ryegrass leaf sheath, or pseudostem, component of the pasture. Ryegrass leaf sheath made up only 5% of the total herbage removed by the E+ sheep compared with 20% of the total herbage removed by the E- sheep. At the time of the trial, non-ryegrass species made up to between 40% and 50% of the

total pasture composition in both the E+ and E- pastures. Sheep grazing E+ pasture selected the other species rather than ryegrass as a greater component of their diet than sheep grazing E- pasture did. Results in Table 8.9. showed that 54% of the total herbage removed by the ewes grazing E+ pasture was non-ryegrass species compared with only 29% by ewes grazing E- pasture. Therefore, not only were ewes grazing E+ pasture eating less total pasture than E- ewes, they were also eating less ryegrass as a proportion of their total diet. This indicates a deliberate avoidance by the ewes of the toxic ryegrass in favour of other non-toxic pasture species. In situations where E+ ryegrass was the only pasture species present, this avoidance of the toxic plant would not be possible. This situation would exacerbate reductions in feed intake by further restricting the proportion of the pasture acceptable to the animal and/or result in a greater incidence and severity of toxicosis as a consequence of animals being forced to consume more of the toxic ryegrass plant.

To date, there have be no published reports examining whether sheep can distinguish between E+ and E- ryegrass when offered simultaneously. However, the current trial indicates that sheep can distinguish between E+ ryegrass and other non-toxic grass species, and appear to be able to distinguish between components within the ryegrass plant of different toxicity.

The mechanisms by which ewes discriminate against the toxic portions of the ryegrass plant are not currently understood. Ruminants select food to minimise unpleasant and maximise pleasant olfactory and other sensations (Arnold & Dudzinski, 1978), so it is possible that the toxic ryegrass may offend one or more of the sheep's senses.

The grazing data clearly demonstrate the greater reluctance of sheep to graze E+ ryegrass, and in particular the pseudostem horizon. This particular reluctance of sheep to graze the pseudostem horizon of the ryegrass pasture could be due to higher endophyte toxin levels in this component of the pasture. Ergovaline and lolitrem B analysis of the various components of the E+ ryegrass (Table 8.7.) plant showed that levels of these toxins were significantly higher in the leaf sheath and stem components of the plant, which make up the pseudostem, than in the leaf blade components. Ergovaline and lolitrem B distribution in the E+ plant in this trial was consistent with other reports that examined the distribution of endophyte toxins in the ryegrass plant during spring (Lane *et al.*, 1997a; Ball *et al.*, 1997). The seasonal nature of endophyte toxin production also appears to be reflected in grazing behaviour. Increasing toxin levels in autumn were associated with an increase in postgrazing herbage mass

differences between E+ and E- pastures (see Chapter III). Differences in pasture composition between autumn and other seasons could also be an important determining factor in reduced feed intake in sheep grazing E+ pasture. The presence of non-ryegrass species within an E+ ryegrass-dominant pasture allow sheep to select these species as part of their diet and thereby dilute the toxin intake associated with the E+ ryegrass. However, many of the non-ryegrass species have disappeared from the sward by the end of summer due to climatic conditions and increased grazing pressure, and ryegrass dominance has increased. With an increase in grazing pressure the opportunity for selection decreases (Chapman & Clark, 1984). Therefore, sheep will have very little dietary choice and are forced to eat greater quantities of toxic ryegrass. This will result in an increase in the toxic effects of endophyte such as ryegrass staggers, and a further reduction in feed intake. Additionally, ryegrass pastures are generally short with a large proportion of pseudostem material present at this time of the year, which exacerbates toxin intake.

The reduced acceptability to grazing sheep of the pseudostem portions of the E+ ryegrass pasture, compared with E- pasture, effectively means a reduction in the pre-grazing herbage allowance of sheep grazing E+ pastures irrespective of whether pre-grazing herbage mass and grazing management are the same for each pasture type. Therefore sheep grazing E+ pasture will have reduced feed intakes and as a result ewe and lamb liveweight will suffer.

4.3. Conclusions

Reduced feed intake is a significant factor in lower liveweight and liveweight gains of ewes and lambs grazing endophyte-infected perennial ryegrass pasture.

Sheep have a greater grazing preference for endophyte-free than endophyte-infected perennial ryegrass and are particularly reluctant to graze the leaf-sheath and stem components of the plant that make up the pseudostem horizon in E+ pastures.

Sheep grazing E+ ryegrass-dominant pasture where other pasture species are present will select these other species as a greater proportion of their diet than sheep grazing E- ryegrass-dominant pasture.

CHAPTER IX

General discussion and conclusions

The objectives of this study were to determine the effects of grazing E+ and E- perennial ryegrass on the major aspects of reproductive performance in ewes. This included close examination of the important reproductive processes associated with the oestrous cycle, gestation, and parturition. Effects on the subsequent lactation and offspring performance were also incorporated into the study, as they are integral parts of the reproductive process. Additionally, in-depth experimental work was undertaken to identify the possible endocrinological and physiological mechanisms associated with endophyte toxin-induced reproductive dysfunction.

1. Reproductive performance of the ewe

The fungal endophytes present in many tall fescue and ryegrass pastures produce a range of compounds that have been implicated as detrimental to grazing animal productivity. Among the more serious detrimental effects commonly reported in animals grazing E+ tall fescue are reduced reproductive rate (number of offspring born per female mated) and reduced milk production. There are many reported similarities between E+ tall fescue and E+ ryegrass in the type of toxins produced and the associated animal toxicoses, however, the effects on reproductive rate and milk production were yet to be fully examined in animals grazing E+ perennial ryegrass.

Eerens *et al.* (1994) found the only significant effect on reproduction in the ewe that could be attributed to grazing E+ ryegrass was a small delay in parturition date. However, this study was conducted in a cool moist environment, which may have reduced or even eliminated some of the possible toxic effects of endophyte. Additionally, this study did not closely examine detailed aspects of ewe reproductive performance such as ovulation rate. Therefore, it was necessary to examine reproductive performance in more detail, in environments that are warmer, and where endophyte toxicosis is considered to be more of a problem.

Results from the grazing trials conducted in the Manawatu (Chapter III) and in Northland (Chapter IV), did not show any significant difference in any of the parameters that determine

reproductive rate (ovulation rate, returns to oestrus and scanning %) between ewes grazing E+ and E- ryegrass pastures in any year of the trials. However, a small delay in mating in the E+ ewes, consistent with the delay in parturition reported by Eerens *et al.* (1994), was observed in the Manawatu trial, although, this is considered to be of little significance to ewe reproductive performance. Data collected from the Northland trial were confounded by the limited control over experimental conditions associated with the large scale and isolation from the researchers conducting the trial.

There were significantly greater numbers of dry ewes for the entire trial period in the Manawatu trial, in the group grazing the E+ pasture compared with those grazing E- pasture, which suggests that lifetime reproductive performance in ewes grazing E+ ryegrass pasture will be lower than ewes grazing E- pasture. This overall depression in reproductive performance may be the result of cumulative effects of the endophyte toxins on the reproductive system and/or chronically lower liveweights in the E+ ewes. A greater number of dry ewes in the E+ group may reflect a higher incidence of fertilisation failure and/or early embryonic mortality. No other reproductive parameters were significantly different between the E+ and E- groups for the entire trial period.

The apparent lack of direct significant effects of grazing E+ ryegrass on ewe reproductive rate is in contrast to reports associated with E+ tall fescue. Reasons for this are unclear at present but may be associated with the comparatively lower levels of toxins that are common to each grass type in the E+ ryegrass, and the presence of other toxins in E+ tall fescue that are not present in E+ ryegrass. The relative differences in toxin levels between the E+ tall fescue and E+ ryegrass brings into consideration the possible presence of toxin threshold levels, above which, the different adverse effects are manifested.

There appear to be differences in sensitivity to endophyte toxins between species of grazing animal, although this may be due to differences in how the toxins are acquired (grazing behaviour) and differences in metabolism of the toxins between ruminants and monogastric animals. However, there are instances where physiological differences between species of grazing animal have been implicated in producing differential effects in endophyte toxicosis. An example of this is the greater severity of depressed milk production in mares grazing E+ tall fescue compared with cattle and sheep. This is largely due to the fact that the mare, unlike the ewe and cow, does not produce placental lactogen, which can alleviate the effects of

depressed prolactin in animals grazing E+ pasture. There may also be differences in sensitivity between physiological systems within a species. Some of the adverse effects associated with grazing E+ tall fescue and E+ ryegrass pastures, such as increased body temperature, increased dag formation, and reduced liveweight gains, were observed in ewes on the grazing trials conducted in this study (Chapters III and IV). However, there was an apparent lack of effects on reproductive rate. This may be indicative of differences in the sensitivity of some physiological systems to the level of endophyte toxins present in these trials. It is possible that the physiological processes controlling the reproductive system require higher toxin levels than those present in the E+ pasture in this trial to provoke adverse effects. The production of many classes of toxin, and variants within a class, by E+ pastures, and the possibility of synergism between toxins, makes it difficult to determine threshold levels for each toxin with respect to physiological manifestations in grazing animals. A significant depression of ovulation rate has been observed in ewes grazing a tall fescue pasture that contained 3 ppm (leaf-blade and sheath were combined) ergovaline at mating (Kramer *et al.,* 1999). Ergovaline levels during mating in the Manawatu and Northland trials were not more than 1 ppm in the ryegrass leaf-blade and not more than 2 ppm in leaf-sheath at any time. Since the leaf-sheath component of the ryegrass plant is generally avoided by the ewe (as demonstrated in Chapter VIII), the toxin level of most interest is that in the leaf blade as this component contributes most to the diet of ewes grazing an E+ ryegrass-dominant pasture. This may suggest that pasture ergovaline levels greater that 1 ppm in the leaf-blade are required to reduce ovulation rate. Levels in excess of 1 ppm have been found in E+ ryegrass pasture (Lane *et al.,* 1997a) and extreme ergovaline production (27 ppm) has been reported in E+ ryegrass under glasshouse conditions (Lane *et al.,* 1997b). Therefore, it is possible that under certain conditions where toxin levels are increased, reproduction could be affected in animals grazing E+ ryegrass pasture.

2. Effects of endophyte toxins on hormones associated with reproduction in the ewe

Although many of the fertility parameters (ovulation rate and conception rate) that determine reproductive rate were not significantly affected in ewes grazing the E+ ryegrass pasture treatments, there were significant differences between the E+ and E- ewes in some of the endocrine systems that regulate reproductive processes. Results in Chapter VI showed that both prolactin and progesterone secretion during the oestrous cycle were significantly affected in ewes grazing E+ ryegrass pasture. The pre-ovulatory prolactin surge was completely

suppressed in ewes grazing E+ pasture. Similarly, blood samples taken from ewes prior to mating in the grazing trials (Chapters III and IV) also showed that serum prolactin was lower in animals grazing E+ pasture compared with those grazing E- pasture. The implications of this depressed prolactin to reproductive rate are unclear. It possible that prolactin has important roles in oestrous activity in the ewe (Kann & Denamur, 1974; Polkowske *et al.*, 1976). However, the extent to which prolactin secretion must be disrupted to affect normal ovarian function is not clear. A reduction in ovulation rate of ewes grazing E+ tall fescue was also associated with an almost total suppression of serum prolactin (Kramer *et al.*, 1999). Similarly, ovulation rate has been reduced in ewes that had serum prolactin levels suppressed by bromocriptine treatment (Rodway *et al.*, 1983). However, Louw *et al.* (1974) concluded that the large quantity of prolactin normally released before oestrus in ewes is not essential for the ovarian changes that occur at this stage of the oestrous cycle. They also concluded that it is possible that small basal amounts of prolactin are sufficient to maintain ovarian function. This brings into question the importance of prolactin suppression in animals grazing E+ pasture in reduced reproductive performance.

It was clear that serum prolactin levels were depressed in ewes grazing the E+ pasture treatments, however, this depression was not as severe as in animals grazing tall fescue. This again raises the question of toxin threshold levels for various effects on hormone secretion. An indoor feeding trial by Cheeke *et al.* (1993) found that very low levels of ergovaline were sufficient to provoke maximum depression of prolactin in sheep. Similarly Fletcher & Easton (1997) confirmed that prolactin depression was characterised by a low threshold to ergovaline, with maximum depression occurring at or below 0.5 ppm in the pasture. Ergovaline levels during mating in the Manawatu and Northland grazing trials were often above 0.5 ppm in the ryegrass leaf-blade, and more than 1 ppm in the leaf-sheath. However, this did not result in the maximal depression suggested in the aforementioned reports. This may be due to the cyclic activity of the ewes in the grazing trials and/or differences in ambient temperature at blood sampling between those in the reports and those in the grazing trial. For whatever reason, the depression of prolactin level prior to mating in the grazing trials was not sufficient to affect fertility in the ewes.

Differences measured in serum progesterone secretion between the ewes grazing E+ and E- pasture during mating (Chapter VI) were small and were associated with a shift in the progesterone secretion profile relative to the day of mating rather than differences in the shape

or amplitude of the secretion profile. Ovulation rates were not measured in these ewes so it cannot be determined whether the progesterone effects were associated with a reduction in fertility. Given the size of the effect on progesterone secretion it seems unlikely that fertility would have been affected.

From the results of the grazing trials (Chapters III and IV), and the examination of hormone levels during the oestrous cycle (Chapter VI), it can be concluded that toxin levels were not sufficient in the E+ ryegrass pastures to significantly reduce reproductive rates. It is unlikely that ewes grazing E+ ryegrass pasture will suffer a reduction in reproductive rate to the extent observed in sheep grazing E+ tall fescue pastures. However, there is the potential that higher toxin levels can occur in E+ ryegrass pastures. The effects on reproductive performance in ewes grazing higher toxin levels in E+ ryegrass has yet to be investigated.

3. Endophytic ryegrass and ram fertility

Investigations on fertility carried out in the present study focussed specifically on the ewe. However, it is possible that fertility may also be affected in rams grazing E+ ryegrass pastures. There have yet to be studies conducted that examine the effects of E+ tall fescue and E+ ryegrass on ram fertility. However, male rats fed E+ tall fescue extracts have exhibited retarded gonadal function and epididymyl development, depressed sperm production and lower testicular weights (Zavos *et al.,* 1986). Bulls grazing E+ tall fescue (Almer & Erickson, 1990) and E+ ryegrass (Bass *et al.,* 1977) have been found to have lower serum testosterone levels. Given the apparent adverse effects of consuming endophytic diets on male fertility in these species, it is possible that rams grazing E+ pastures may also suffer in a similar manner.

In the Manawatu trial the rams used were grazed year round on E+ ryegrass pastures, but they were rotated weekly between the E+ and E- groups during mating to eliminate possible differences in ram fertility resulting from the endophyte treatments. Adverse effects on ram fertility could potentially have a greater impact on overall flock performance as reduced fertility in one ram can have negative effects on the subsequent reproductive performance of many ewes. The importance of ram fertility in reproductive performance of sheep flocks makes it necessary to investigate the possible adverse effects of grazing E+ ryegrass pastures.

4. Ewe liveweight, lamb growth rate, and feed intake

Lower liveweight and growth rates in sheep grazing E+ ryegrass pastures have been previously reported (Fletcher *et al.*, 1999). There were significant differences in ewe liveweight and lamb growth rate in both the Manawatu and the Northland (Chapters III, and V) grazing trials when the treatment groups were given the same pasture allowance and grazing management. The lower liveweight and liveweight gain in animals grazing E+ ryegrass pastures has serious implications for reproductive performance. Poor ewe liveweight and liveweight gain during mating have a well defined relationship with reduced reproductive rates (Morley *et al.*, 1978). Therefore, it is likely that poor liveweight performance in ewes grazing E+ ryegrass will cause reductions in reproductive rate. Differences in ewe liveweight between the E+ and E- ewes in the Manawatu grazing trial did not result in significant differences in reproductive rate. However, ewe liveweight was a significant source of variation in ovulation rate and scanning % within the E+ group. This suggests that lower liveweight as a result of grazing E+ pasture limited the reproductive rate within the E+ group. There was no significant difference in liveweight between ewes of different reproductive rate in the E- group. This was probably due to the greater average liveweight within the group and, therefore, liveweight was not limiting reproductive rate to the extent that it was within the E+ group. With the differences in ewe liveweight measured in these trials and the number of ewes used in each group, it is unlikely that significant differences in reproductive rates will be measured within each year. However, chronically lower liveweight is likely to reduce the ewe's lifetime reproductive performance, as indicated by the higher number of dry ewes in the E+ group in the Manawatu trial, and is, therefore, an important consideration in evaluating the effects of grazing E+ pasture.

Supply of E- pasture was often limited in comparison to the E+ pasture during mating in the Manawatu and Northland trials. This may have been due to lower post-grazing residues over the summer period, and insect attack in the Northland trial in 1997. This reduction in pasture supply resulted in weight loss in the E- ewes during mating. As the effects of ewe liveweight on reproductive performance are both static (liveweight at mating) and dynamic (liveweight gain prior to and during mating), the positive effects of greater mating liveweight on reproductive rate in the E- group may have been confounded by the liveweight loss. To alleviate this problem, grazing management of E- pastures should be different from E+

pastures to ensure that adequate feed is available during autumn for mating. This will ensure that the reproductive benefits of better liveweight in the E- ewes can be realised.

Results from the Manawatu grazing trial and the lamb growth rate trial conducted in Northland (Chapter V) showed that lamb growth rates and weaning weights are better on E- than on E+ pastures. Lamb liveweight gain has been linked to the attainment of puberty and subsequent reproductive performance (Dyrmundsson, 1973; Keane, 1974; Hawker, 1977). Although, data on the attainment of puberty in the E+ and E- lambs was not measured, it is likely that the E- lambs would reach puberty earlier.

Given the importance of ewe liveweight and lamb growth rate to reproductive performance, it was necessary to identify the factor(s) responsible for the differences in these parameters between sheep grazing E+ and E- pasture. Fletcher & Barrell (1984) speculated that a reduced feed intake was responsible for the poorer liveweight of sheep grazing E+ pastures. Additionally, differences in post grazing residuals between E+ and E- pastures grazed by ewes described in Chapter III (and also by Edwards *et al.*, 1993), are indicative of higher feed intakes on the E- pasture. Results obtained in Chapter VIII showed that higher feed intake by the ewes and lambs in the E- group compared with E+ caused differences in post-grazing residues. Using chromium tracer as an indicator of feed intake, it was apparent that both the ewes and lambs in the E+ group had significantly lower herbage intakes than the ewes and lambs in the E- group during late lactation (15-18 weeks *postpartum*). It was also apparent that differences in feed intake were related to differences in liveweight and liveweight gain in both the ewes and lambs. The reason for this reduction in feed intake appeared to be an aversion by the E+ ewes and lambs to consuming the toxic E+ ryegrass plant and in particular the leaf-sheath component where toxin levels are generally greater.

5. Grazing behaviour and pasture composition

The reluctance of ewes and lambs to graze E+ ryegrass, described in Chapter VIII, has obvious implications for feed intake where E+ is 100% of the pasture composition, in that, feed intake will be lower on E+ pastures when no other non-toxic forage species is present to be consumed by the animal. Contamination of the E+ and E- pastures by non-*Lolium* grass and by clovers in the grazing trial described in Chapter VIII provided useful information on dietary selection of sheep grazing pastures where either E+ or E- ryegrass was the dominant

species. Results showed clearly defined differences in dietary preference for the E+ and E- ryegrass. Ryegrass was a significantly greater proportion of the diet of sheep grazing the E- pasture compared with those grazing the E+ pasture. This demonstrated a deliberate avoidance of E+ ryegrass in preference for the other pasture species. Given this apparent difference in grazing behaviour, it follows that forage species composition in mixed ryegrass/non-ryegrass pastures will be differentially affected according to whether the ryegrass is endophyte-infected or endophyte-free.

Most ryegrass pastures sown in New Zealand have white clover in the seed mix (Lancashire, 1984). The effects of the endophyte status of ryegrass used in ryegrass/white clover mixes on the performance of the accompanying white clover have been investigated previously. In pastures where white clover was sown with E+ ryegrass it was found that fewer clover seedlings survived, an inverse competitive relationship existed between grass yield and clover survival, and there was greater reduction in the amount of clover in the E+ pasture after grazing compared with the E- pasture (Sutherland & Hoglund, 1989). This study concluded that the major factors affecting clover survival in the E+ pasture were increased competition by the grass, defoliation by the animal and an unexplained allelopathic effect. Prestige *et al.* (1992) found that there were no differences in clover content between E+ and E- pasture when ryegrass tiller densities were similar. However, after grazing, the tiller densities of the E- plants decreased relative to E+ plants and there was a strong inverse relationship between ryegrass tiller density and white clover vigour and growth. These researchers hypothesised that below-ground competition between ryegrass and white clover had the greatest influence on white clover vigour and growth, and that allelochemicals released by E+ ryegrass had no direct influence on this relationship.

Both the previously mentioned studies report a difference in the ryegrass to clover ratio between the E+ and E- pastures. They both conclude that reasons for this appear largely to be due to greater competition by the E+ ryegrass compared with the E- ryegrass rather than the result of differences in defoliation patterns of the grazing animal. This conclusion is perhaps due to the fact that clover content was lower in E+ pastures compared with E- pastures even when defoliation was done with a mower.

The difference in grazing behaviour, specifically diet selection, described in Chapter VIII could be a major factor in causing differences in the ryegrass to clover ratio between the E+

and E- pastures. Milne *et al.* (1982) showed that clover is selected by grazing animals in preference to ryegrass. However, it is evident that the use of E- ryegrass may reduce the preference for clover over ryegrass, whereas the use of E+ ryegrass will increase the preference for clover due to a greater propensity of the sheep to avoid the E+ ryegrass. These differences in the relative preference for each component between the E+ and E- pastures could explain the higher clover content of E- pastures i.e. more ryegrass is being consumed leaving more clover. Conversely, the lower clover content in the E+ pastures is associated with a greater preference for clover compared to ryegrass.

The benefits of increased clover content in pasture are well understood. Increased white clover content in ryegrass pastures results in improved ewe and lamb liveweight gains (Gibb & Treacher, 1982), and improved nitrogen fixation for plant growth (Harris & Hoglund, 1980). The benefits to animal performance of increased clover content compound the existing benefits of E- ryegrass compared with E+ ryegrass.

Although feed intake appears to be a major determining factor in reduced liveweight in ewes grazing E+ pasture, there are other factors that should not be ignored. It is possible that endophyte toxins affect growth hormone and insulin levels (Browning *et al.*, 1997: Oliver, 1997), and gastrointestinal smooth muscle motility (Smith *et al.*, 1997) to the extent that nutrient metabolism and uptake may also be adversely affected.

6. Milk production, mammary development and maternal behaviour

Milk production, growth rate, and health of the offspring are integral parts of reproductive performance. Milking data from Chapter III indicated that there were differences in milk production between ewes grazing E+ and E- pasture. This may be responsible for the differences in lamb growth rate between treatments. Reduced milk production has been reported in sheep grazing tall fescue but had yet to be examined in sheep grazing E+ ryegrass. Cows grazing E+ ryegrass have lower milk yields than cows grazing E- ryegrass, which may be due to a reduction in feed intake (Blackwell & Keogh, 1999). Given that differences in feed intake and liveweight were measured between the E+ and E- groups, it is likely that this would result in differences in milk production, and subsequent growth of their lambs. Differences in lamb growth between the E+ and E- groups were greatest during early lactation (1 – 6 weeks *post partum*) when milk is a higher proportion of the lambs' diet. This indicated

that milk production differences between the groups of ewes might have been more evident during this time. As lactation progressed, any effects the differences in milk production between the E+ and E- ewes may have had on lamb growth rate would have been complicated by differences in feed intake between the E+ and E- lambs.

In addition to reduced feed intake and ewe liveweight, it is possible that the disruption of hormones regulating mammary gland development and lactogenesis by endophyte toxins may also affect milk production in the ewe. Results in Chapter VII showed that it required high ergovaline levels (5 ppm) in the diet and high ambient temperature (30°C) to completely abolish mammary development and milk production. This was associated with marked effects on the levels of serum prolactin and progesterone in the treated ewes. However, ewes that were fed diets containing ergovaline at lower temperature (18°C) did not appear to suffer any obvious adverse effects on mammary development despite reduced serum progesterone levels. There were pronounced differences in maternal behaviour between the Ev+ and Ev- groups at both temperature treatments, which suggests that the hormonal control of dam/offspring behaviour after parturition was disrupted by ergovaline. As most flocks in New Zealand lamb in early spring, this combination of high toxin levels and high ambient temperature is generally not likely to be present during late pregnancy and parturition when the processes of mammary development and maternal bonding are carried out. Therefore, it is unlikely that the effects of endophyte toxins on hormones regulating these processes will be of major concern in the spring-lambing ewe. However, it may have implications for autumn-lambing ewes and for ewe lambs attaining puberty when mammary development will be taking place during high summer temperatures and high pasture toxin levels.

7. Conclusions

From the results obtained in the present study it can be concluded that levels of endophyte toxins that are normally present in E+ perennial ryegrass pastures throughout the year are not sufficient to provoke significant acute reductions in reproductive rate similar to those observed in E+ tall fescue. However, there is likely to be lower life-time productivity, including that associated with reproduction, by ewes grazing E+ ryegrass pastures that may largely be associated with lower feed intakes and subsequent effects on ewe condition. The effects of grazing E+ ryegrass pasture on feed intake and condition of ewes may also be reflected in poorer performance of lambs.

The quandary facing the New Zealand sheep farmer is that gains in animal performance through using E- ryegrass will often be offset by poor pasture persistence, particularly in climates where plants are under greater threat from insect attack and drought. These problems were clearly demonstrated in the Northland trial (Chapters IV, 1998 trial), where any gains that may have resulted from grazing the E- pasture were reduced by poor pasture production. The benefits that can be achieved in animal performance by not grazing E+ pasture certainly should be pursued. However, alternatives to E- ryegrass, such as other non-endophytic grass species, may be necessary for some regions of New Zealand. Alternatively, the answer may lie in the use of ryegrass plants infected with different strains of endophyte that confer insect resistance but do not produce the major toxins responsible for animal disorders.

Appendix I

Ewe deaths in the Manawatu grazing trial during 1997 and 1998

Appendix Table 1.1. Number and cause of ewe deaths in the E+ and E- groups during 1997 and 1998 in the Manawatu trial.

NUMBER OF EWES	CAUSE OF DEATH
	E+
1	Pregnancy toxaemia
2	Euthanasia (prolapsed uterus)
1	Acute mastitis with bacterial septicaemia
1	Severe verminous pneumonia
	E-
1	Dystocia and pregnancy toxaemia
1	Pregnancy toxaemia
2	Euthanasia (prolapsed uterus)

NB: Pathology reports for E+ ewes (p 202-203) and E- ewes (p 204-205) are attached.

Department of Veterinary Pathology and Public Health

PATHOLOGY REPORT

MASSEY
UNIVERSITY

Private Bag
Palmerston North
New Zealand
Telephone 0-6-356 9099
Facsimile 0-6-350 5636

FACULTY OF
VETERINARY
SCIENCE

DEPARTMENT OF
VETERINARY
PATHOLOGY AND
PUBLIC HEALTH

ubmtrs.Ref.:	:Date sent: 19.9.97	:Accn. No.: 28384

):
Reg Keugh
AgResearch
Palmerston North

Species: Ovine	:Sex: Female	:Age:	:Breed: Romney
D: Red 0336	:At risk:	:Affected:	:Dead:
Owner: AgResearch	Reg Keugh Extn 8031	:Prev.Accn.:	:Biopsy: NO

HISTORY: Found dead 19.9.97.

GROSS FINDINGS: The sheep was severely autolysed, with blue/green skin and decomposing organs.
There was extensive subcutaneous oedema and bruising on the ventral surface of the anterior neck.
The liver contained multiple, discrete hard nodules throughout. They were small, 2 mm diameter round nodules with a grey/black colour. On cut surface, the nodules contained black firm material surrounded by fibrous tissue.
The left quarter of the udder was very firm and was considerably swollen compared to the right quarter.
70% of the quarter contained large coalescing areas of yellow caseous necrosis surrounded by zones of hyperaemia.

HISTOPATH: Only the mammary gland and liver were examined histologically. The mammary gland has multiple coalescing areas of caseous necrosis composed of necrotic debris, extremely large numbers of rod-shaped bacteria, often in micro colonies, fibrin and large numbers of neutrophils. Surrounding these areas are a zone of hypertrophied macrophages with proliferating fibroblasts and dilated capillaries.
The focal liver lesions are made up of areas of caseous necrosis and large micro colonies of rod-shaped bacteria morphologically similar to those in the mammary gland. The liver is extensively autolysed and most sinusoids contain very large rods.

Morphological Diagnosis - Severe subacute necrotising mastitis.
Focal necrotising hepatitis.

MICRO.PARAS:

DIAGNOSIS: Acute mastitis with bacterial septicaemia.

COMMENT: The morphology of the bacteria and the nature of the lesion suggest that *Pasteurella haemolytica* is the most likely cause.

File Nos.: 1900/1

Students: Matt Hart, Ivan Holloway, Lyn Graham.

Date: 24 September 1997

:Pathologist: J S Lumsden:

WG

MASSEY
UNIVERSITY

Private Bag 11222
Palmerston North
New Zealand
Telephone +64-6-356 9099
Facsimile +64-6-350 5714

COLLEGE OF
SCIENCES

INSTITUTE OF
VETERINARY, ANIMAL &
BIOMEDICAL SCIENCES

Institute of Veterinary, Animal and Biomedical Sciences

PATHOLOGY REPORT

ubmtrs.Ref.:	:Date sent: 3.8.98	:Accn. No.: 29418

D: M McDonald
Comparative Physiology & Anatomy, IVABS
College of Sciences
Massey University
Palmerston North

Species: Ovine	:Sex: Female	:Age: Full mouth	:Breed: Romney
ID: 0322	:At risk:	:Affected:	:Dead:
:Owner: AgResearch	C/- R Kramer PhD Students, IVABS	:Prev.Accn.:	:Biopsy: NO

:HISTORY: Found dead (lambed 30.7.98).

:GROSS FINDINGS: There was bilateral mucoid nasal discharge. There were large quantities of froth, mucopurulent material and many cream-white worms measuring up to 50 mm length, in the trachea, bronchi and bronchioles.
There were extensive fibrous adhesions between the lungs and pleura and lungs and pericardium, especially on the right hand side. Many of these pleural adhesions were spongy and gaseous.
The lungs were wet and glistening, were heavy and lumpy and contained multifocal areas of dark red firm consolidation throughout the parenchyma, which oozed a mucopurulent exudate on cut surface.
There were three *Cysticercus tenuicollis* in the abdominal cavity attached to the omentum.
The gall bladder was large. The right lobe of the liver was thickened, enlarged and swollen. The caudate lobe was slightly enlarged around the tip. The left liver lobe was pale pink, very thin and almost non-existent (boxing glove liver). The uterus appeared to be involuting normally.

:HISTOPATH:
Morphological Diagnosis
Severe multifocal parasitic (lungworm) bronchopneumonia with adhesions to the pleura.
Chronic atrophy of the left lobe and hypertrophy of the right and middle lobes of the liver.

:MICRO.PARAS:

:DIAGNOSIS: Severe verminous pneumonia (liver shows lesions of chronic facial eczema).

:COMMENT:

:File Nos.:

:Students: Angela Carlaw.

:Date: 6 August 1998

Pathologist: M G Collett:

/WG

Institute of Veterinary, Animal and Biomedical Sciences

PATHOLOGY REPORT

MASSEY
UNIVERSITY

Private Bag 11222
Palmerston North
New Zealand
Telephone +64-6-356 9099
Facsimile +64-6-350 5714

COLLEGE OF
SCIENCES

INSTITUTE OF
VETERINARY, ANIMAL &
BIOMEDICAL SCIENCES

ɔmtrs.Ref.:	:Date sent: 14.7.98	:Accn. No.: 29338

M McDonald
Comparative Physiology & Anatomy, IVABS
College of Sciences
Massey University
Palmerston North

ɔecies: Ovine	:Sex: Female	:Age: 4 years	:Breed: Romney
): No. 358	:At risk:	:Affected:	:Dead:
wner: AgResearch		:Prev.Accn.:	:Biopsy: NO

[ISTORY: Found down yesterday. Able to rise but very weak. Died during the night. Untreated.

;ROSS FINDINGS: The ewe was in very good condition (BCS approximately 4). There was slight overgrowth `the horn of the hooves. A small amount of blood-tinged membrane was seen protruding from the vulva, with blood-tinged perineal area.
he lungs were dark red and congested, and there was a small (2 x 2 cm) area of pleural adhesion to the left chest all.
Vithin the abdomen, the uterus was very large. Two near-term slightly autolysing lambs were present, with the ead of one lodged in the birth canal, with its front legs back.
he rumen was completely empty, with a pH of 8. The rest of the gut was also empty and contained a small nount of gas.
he liver was enlarged, pale, with rounded edges and was friable, and the gall bladder was enlarged.

IISTOPATH:

MICRO.PARAS:

)IAGNOSIS:
1. Dystocia.
2. Pregnancy toxaemia.

COMMENT:

ile Nos.:

tudents: Mark Hosking, Kim Sullivan, Ginny Driscoll.

)ate: 4 August 1998

Pathologist: J S Lumsden / C M Twentyman:

G

Institute of Veterinary, Animal and Biomedical Sciences

PATHOLOGY REPORT

MASSEY
UNIVERSITY

Private Bag 11222
Palmerston North
New Zealand
Telephone +64-6-356 9099
Facsimile +64-6-350 5714

COLLEGE OF
SCIENCES

INSTITUTE OF
VETERINARY, ANIMAL &
BIOMEDICAL SCIENCES

)mtrs.Ref.:	:Date sent: 15.7.98	:Accn. No.: 29344

M McDonald
Comparative Physiology & Anatomy, IVABS
College of Sciences
Massey University
Palmerston North

)ecies: Ovine	:Sex: Female	:Age: 4-Tooth	:Breed: Romney
):	:At risk:	:Affected:	:Dead:
wner: AgResearch		:Prev.Accn.:	:Biopsy: NO

HISTORY: Found dead.

GROSS FINDINGS: The ewe had excessive body fat reserves. The uterus contained two near-term lambs, both which were stained yellow. Urine was collected and when analysed it was shown to contain ketones and had pH of 5-6. The rumen, abomasum and intestines were empty. The liver was fatty, friable and had rounded argins.

HISTOPATH:

MICRO.PARAS:

DIAGNOSIS: Pregnancy toxaemia.

COMMENT:

File Nos.:

Students:

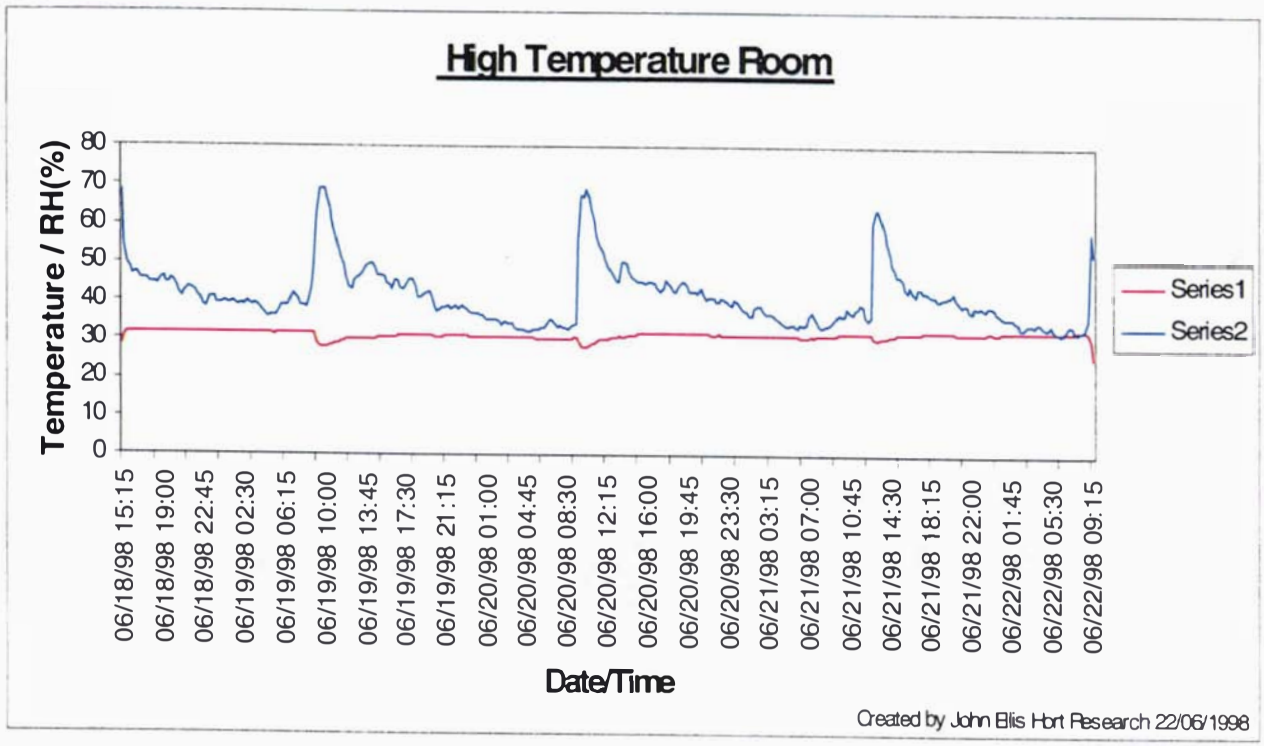
Date: 22 July 1998

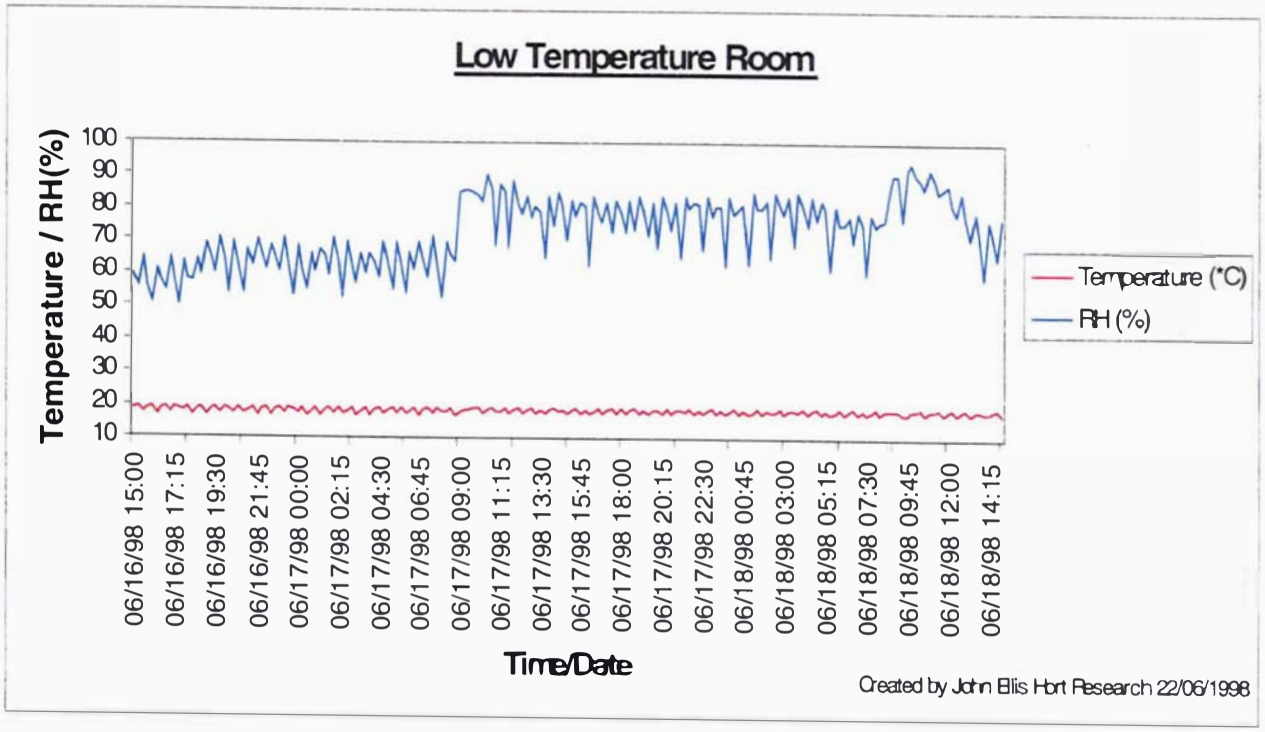
Pathologist: J S Lumsden / C M Twentyman:

/G

Appendix II

Daily fluctuation in ambient temperature and humidity in the high and low temperature rooms in Trial 2 (Chapter VII)





REFERENCES

Achmadi, J.; Yanagisawa, T.; Sano, H.; Terashima, Y. 1993. Pancreatic insulin secretory response and insulin action in heat-exposed sheep given a concentrate or roughage diet. *Domestic Animal Endocrinology* **10:** 279-287.

Alamer, M.A.; Erickson, B.H. 1990. Effect of fungus-infested fescue on testicular development and hormonal secretion in the beef bull. *Journal of Animal Science* **68**(Suppl. 1):402 (Abstr.).

Aldrich, C.G.; Rhodes, M.T.; Miner, J.L.; Kerley, M.S.; Paterson, J.A. 1993a. The effects of endophyte-infected tall fescue consumption and the use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. *Journal of Animal Science* **71**: 158-163.

Aldrich, CG; Paterson, J.A.; Tate, J.L. and Kerley, M.S. 1993b. The effects of endophyte-infected tall fescue consumption on diet utilisation and thermal regulation in cattle. *Journal of Animal Science* **71**: 164-170.

Arnold, G.W.; Dudzinski, M.L. 1978. Diet selection and food intake. In Ethology of free-ranging domestic animals. Developments in animals and veterinary sciences 2. Elsevier Scientific Publishing Company, Amsterdam.

Bacon, C.W.; Porter, J.K.; Robbins, J.D.; Luttrell, E.S. 1977. *Epichloe typhina* from toxic tall fescue grasses. *Applied and Environmental Microbiology* **34**: 576-581.

Badia, A.; Moron, A.; Cuffi, L.; Vila, E. 1988. Effects of ergotamine on cardiovascular catecholamine receptors in the pithed rat. *General Pharmacology* **19**: 475-481.

Ball, O.J.P.; Lane, G.A.; Prestidge, R.A.; Popay, A.J. 1995. *Acremonium lolii*, ergovaline and peramine production in endophyte-infected perennial ryegrass. Proceedings of the Forty Eighth New Zealand Plant Protection Conference, Angus Inn, Hastings, New Zealand, August 8-10: 224-228.

Ball, O.J.P.; Barker, G.M.; Prestidge, R.A.; Sprosen, J.M. 1997. Distribution and accumulation of the mycotoxin lolitrem B in *Neotyphodium lolii*-infected perennial ryegrass. *Journal of Chemical Ecology* **23**: 5: 1435-1449.

Barenton, B.; Pelletier, J. 1980. Prolactin, testicular growth and LH receptors in the ram following light and 2-Bromo-α-Ergocryptine (CB 154) treatments. *Biology of Reproduction* **22**:781-790.

Barker, D.J.; Davies, E.; Lane, G.A.; Latch, G.C.M.; Nott, H.M.; Tapper, B.A. 1993. Effect of water deficit on alkaloid concentrations in perennial ryegrass endophyte associations. Hume, D.E.; Latch, G.C.M. and Easton, H.S. (Eds), Proceedings of the Second Symposium on *Acremonium*/Grass Interactions. 67-71.

Barnett, D.T. 1985. Fescue toxicosis update. University of Kentucky Equine Data Line. September EL-1. Lexington, KY.

Barth, K.M.; Jordan, M.A.; Keltner, D.G.; McLaren, J.B.; Fribourg, H.A. 1991. Effect of Acremoniun coenophialum infestation of tall fescue on forage digestibility and selectivity by beef steers. *Tennessee Farm and Home Science* **160**: 54-59.

Bass, J.J.; Peterson, A.J.; Byford, M. 1977. The effect of ryegrass staggers on plasma testosterone in bulls. New Zealand Ministry of Agriculture and Fisheries: Agricultural Research in the New Zealand Ministry of Agriculture and Fisheries Annual Report of Research Division 1975-76, 60.

Bassett, J.M.; Oxborrow, T.S.; Smith, I.D.; Thorburn, G.D. 1969 The concentration of progesterone in the peripheral plasma of the pregnant ewe. *Journal of Endocrinology* **45**: 449-457.

Belo, C.C. 1990. Forage quality and environmental temperature influence on energy partitioning for milk production and composition in sheep. *Dissertation Abstracts International. B, Sciences and Engineering* **50**: 4830B.

Bendicho de Combellas, J.; Martinez, N.D.; Gonzalez, J.E. 1979. A study of some factors affecting lamb body weight at birth and weaning. *Memoria Asociacion Latinoamericana de Produccion Animal* **14**: 136-137.

Berde, B.; Schild, H.O. 1978. The ergot alkaloids and related compounds. In: Handbook of Experimental Pharmacology. Vol. 49. Springer-Verlag, New York.

Bevers, M.M.; Dieleman, S.J.; Kruip, T.A.M.; Willemse, A.H. 1985. Follicular development in heifers chronically treated with bromocryptine. Follicular growth and ovulation rate in farm animals. A seminar in the CEC Programme of Co-ordination of Research in Animal Husbandry, Dublin, Ireland. p. 45-53.

Berman, A. 1991. Reproductive responses under high temperature conditions. *Animal Husbandry in Warm Climates.* EAAP Publication **55**: 23-30.

Blackwell, M.B.; Keogh, R.G. 1999. Endophyte toxins and performance of spring-calving cows in Northland. *Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series* No. 7:45-50.

Blank, R.H.; Bell, D.S.; Olson, M.H. 1986. Differentiating between black field cricket and black beetle damage in Northland pastures under drought conditions. *New Zealand Journal of Experimental Agriculture* **14**: 3, 361-367.

Blank, R.H.; Chapman, R.B. 1985. (ed.)Black beetle or black field cricket - the insect villain of pastures in Northland?. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology, held at Lincoln College, University College of Agriculture, Canterbury, New Zealand, 13-17 May, 1985. 1985, 344-352.

Blank, R.H.; Olson, M.H. 1987.Screening sixteen pasture species for tolerance to attack by black field cricket (*Teleogryllus commodus*). *New Zealand Journal of Experimental Agriculture* **15**: 87-90.

Blundell, J. 1991. Pharmacological approaches to appetite suppression. *Trends in Pharmacology Science* **12**: 147-157.

Blythe, L.L.; Tor -Agbidye, J.; Craig-AM. 1993. Correlation of quantities of lolitrem B toxin to clinical field cases of ryegrass staggers. *New Zealand Veterinary Journal* **41**: 4, 217

Boling, J.A. 1985. Endophytic fungus and tall fescue utilisation by ruminants *Professional Animal Science* **1**:19.

Bolt, D.J.; Bond, J.; Lynch, G.P.; Elsasser, T. 1982. Concentrations of PRL, LH, FSH, GH, and TSH in plasma and pituitary of ewes grazing tall fescue and orchardgrass pastures. *Journal of Animal Science* **55**(Suppl 1):4 (Abstr.)

Bond, J.: Bolt, D.J. 1986. Growth, plasma prolactin, and ovarian activity in heifers grazing fungus-infected tall fescue. *Nutrition Reports International* **34**:93-102.

Bond, J.; Hawk, H.W.; Lynch, G.P.; Jackson, C. 1982. Lower fertility in ewes grazing tall fescue pastures. *Journal of Animal Science* **55**(Suppl. 1): 46 (Abstr.)

Bond, J.; Lynch, G.P.; Bolt, D.J.; Hawk, H.W.; Jackson, C.; Wall, R.J. 1988. Reproductive performance and lamb weight gains for ewes grazing fungus-infected tall fescue. *Nutrition Reports International* **37**:1099.

Brendemuehl, J.P.; Williams, M.A.; Boosinger, T.R.; Ruffin, D.G. 1994a. Plasma progestagen, tri-iodothyronine, and cortisol concentrations in post-date gestation foals exposed in utero to the fescue endophyte *Acremonium coenophialum.* Proceedings of the 6th International Symposium on Equine Reproduction P. 11-12.

Brendemuehl, J.P.; Boosinger, T.R.; Shelby, R.A. 1994b. Influence of endophyte-infected tall fescue on cyclicity, pregnancy rate and early embryonic loss in the mare. *Theriogenology* **42**: 489-500.

Brown, D.E.; Harrison, P.C.; Hinds, F.C.; Lewis, J.A.; Wallace, M.H. 1977. Heat stress effects on fetal development during late gestation in the ewe. *Journal of Animal Science* **44**: 442-446.

Browning, R.; Tompson, F.N.; Sartin, J.L.; Leite-Browning, M.L. 1997. Plasma concentrations of prolactin, growth hormone, and luteinizing hormone in steers administered ergotamine or ergonovine. *Journal of Animal Science* **75**: 796-802.

Browning, R.; Leite-Browning, M.L. 1997. Effect of ergotamine and ergonovine on thermal regulation and cardiovascular function in cattle. *Journal of Animal Science* **75**: 176-181.

Browning, R.; Leite-Browning, M.L.; Smith, H.M.; Wakefield. T. Jr. 1998. Effect of ergotamine and ergonovine on plasma concentrations of thyroid hormones and cortisol in cattle. *Journal of Animal Science* **76**: 6, 1644-1650.

Burd, L.I.; Takahashi, K.; Ward, K.; Ascherman, G.; Dowers, S.; Scommegna, A. 1978. The relationship of changes in mammary blood flow and plasma progesterone at the time of parturition in the ewe. *American Journal of Obstetrics and Gynecology* **132**: 385-391.

Buys, N.; Peeters, R.; De Clerck, B.; Van Isterdael, J. Kuhn, E.R.; Decuypere, E. 1990. Seasonal variations in prolactin, growth hormone and thyroid hormones and the prolactin surge at ovulation do not affect litter size of ewes during pregnancy in the oestrous or the anoestrous season. *Journal of Reproduction and Fertility* **90**: 47-53.

Chamley, W.A.; Buckmaster, J.M.; Cerini, M.E.; Cumming, I.A.; Goding, J.R.; Obst, J.M.; Williams, A.; Winfield, C. 1973. Changes in the levels of progesterone, corticosteroids, estrone, estradiol-17β, luteinizing hormone and prolactin in the peripheral plasma of the ewe during late pregnancy and at parturition. *Biology of Reproduction* **9**: 30-35.

Chandrashekra, V.; Bartke, A.; Sellers, K. 1987. Prolactin modulates the gonadotropin response to the negative feedback effects of testosterone in immature male rats. *Endocrinology* **120**: 758-763.

Chapman, D.F.; Clark, D.A. 1984. Pasture responses to grazing management in hill country. *Proceedings of the New Zealand Grassland Association* **45**: 168-176.

Cheeke, P.R.; Luick, B.R.; Debessai, W. 1993. Effects of feeding endophyte infected tall fescue seed on lamb performance and serum prolactin. *New Zealand Veterinary Journal*. 214.

Chestnut, A.B.; Bernard, J.K.; Harstin, J.B.; Reddick, B.B. 1992. Performance of growing lambs fed *Acremonium coenophialum* infested tall fescue (*Festuca arundinacea* Schreb.) hay. *Small Ruminant Research* **7**: 9-19.

Christopher, G.K.,; Salfen, B.E.; Schmidt, S.P.; Arbona, J.R.; Marple, D.N.; Sartin, J.L.; Bransby, D.I.; Carson, R.L.; Rahe, C.H. 1990. Effects of grazing Kentucky-31 tall fescue infected with *Acremonium coenophialum* on endocrine function in ovariectomized beef heifers. *Journal of Animal Science* **68**(Suppl. 1): 469 (Abstr.).

Clark, D.A.; Thom, E.R.; Waugh, C.D. 1996. Milk production from pastures and pasture silage with different levels of endophyte infection. *Proceedings of the New Zealand Society of Animal Production* **56**: 292-296.

Clark, D.A.; Thom, E.R.; Waugh, C.D.; Burggraaf, V.T. 1999. Milk production from perennial ryegrass pastures containing different levels of endophyte. *Proceedings of the New Zealand Society of Animal Production* **59**: 258-259.

Clifton, D.K.; Sawyer, C.H. 1980. Positive and negative feedback effects of ovarian steroids on luteinizing hormone release in ovariectomized rats following chronic depletion of hypothalamic norepinephrine. *Endocrinology* **106**: 1099-1102.

Cockrem, F.R.M.; McDonald, M.F. 1969. An investigation of the relationships between body temperature and implantation and lambing rates in the New Zealand Romney ewe. *Proceedings of the New Zealand Society of Animal Production* **29**:195-207.

Coen, C.W.; Franklin, M.; Laynes, R.W.; MacKinnon, P.C.B. 1980. Effect of manipulating serotonin on the incidence of ovulation rate in the rat. *Journal of Endocinology* **87**: 195-201.

Coffey, K.P.; Moyer, J.L.; Brazle, F.K.; Lomas, L.W. 1992. Amount and diurnal distribution of grazing time by stocker cattle under different tall fescue management strategies. *Applied Animal Behaviour Science* **33**: 121-135.

Cohen, M.L.; Johnson, M.P.; Schenck, K.W.; Susemichel, A.; Wainscott, D.B.; Rohertson, D.W.; Nelson, D.L. 1993. DOI and α-methylserotonin: comparative vascular and nonvascular smooth muscle effects and central 5-hydroxytryptamine$_2$ receptor affinities. *Journal of Pharmacology and Experimental Therapy* **266**: 93-949.

Cooper, J.R.; Bloom, F.E.; Roth, R.H. 1991. Biochemical Basis of Neuropharmacology (6th Ed). Oxford University Press, New York.

Cosgrove, G.P.; Anderson, C.B.; Berquist, T.R.N. 1996. Fungal endophyte effects on intake, health and liveweight gain of grazing cattle. *Proceedings of the New Zealand Grasslands Association* **57**: 43-48.

Cottam, Y.H.; Peterson, S.W.; McCutcheon, S.N. 1997. Control of reproductive success in rabbits using bromocriptine. *Proceedings of the New Zealand Society of Animal Production* **57**: 222-224.

Cowie, A.T.; Tindal, J.S. 1971. The physiology of lactation. Edward Arnold (Publishers) Ltd, London.

Crawford, R.J.; Forwood, J.R.; Belyea, R.L.; Gardner, G.B. 1989. Relationship between level of endophyte infection and cattle gains on tall fescue. *Journal of Production Agriculture* **2**: 147.

Cross, D.L.; Redmond, L.M.; Strickland, J.R. 1995. Equine fescue toxicosis: signs and solutions. *Journal of Animal Science* **73**: 899-908.

Cross, D.L. 1997. Fescue toxicosis in horses. Proceedings of the International Symposium on *Neotyphodium*/Grass Interactions, Bacon, C.W. and Hill, N.S. (Eds). Plenum Press, New York p. 289-309.

Cunningham, I.J. 1958. Non-toxicity to animals of ryegrass endophyte and other endophytic fungi of New Zealand grasses. *New Zealand Journal of Agricultural Research* **1**: 489-497.

Cunningham, I.J.; Hartley, W.J. 1959. Ryegrass staggers. *New Zealand Veterinary Journal* **7**: 1-7.

Daily, R.A.; Tsou, R.C.; Tindall, G.T.; Neill, J.D. 1978. Direct hypophysial inhibition of luteinizing hormone release by dopamine in the rabbit. *Life Science* **22**: 1491-1498.

Daniels, L.B.; Nelson, T.S.; Beasley, J.N. 1981. Effects of extracts of toxic fescue given orally to rats. *Canadian Journal of Comparative Medicine* **45**:173-176.

Daniels, L.B.; Ahmed, A.; Nelson, T.S.; Beasley, J.N. 1984. Physiological responses in pregnant white rabbits given a chemical extract of toxic tall fescue. *Nutrition Reports International.* **29**: 505-510.

Daunt, D.A.; Maze, M. 1992. Alpha-2 adrenergic agonist receptors, sites and mechanisms of action. In C.E. Short and A.V. Poznak (Eds). Animal Pain. Churchill Livingston, New York p. 165-180.

Deaver, D.R.; Daily, R.A. 1982. Effects of dopamine, norepinephrine and serotonin on plasma concentration of luteinizing hormone and prolactin in ovariectomized and anestrous ewes. *Biology of Reproduction* **27**: 624-632.

Deaver, D.R.; Daily, R.A. 1983. Effects of dopamine and serotonin on concentrations of luteinizing hormone and oestradiol-17 beta in plasma of cycling ewes. *Biology of Reproduction* **28**: 870-877.

Debessai, W.; Luick, B.R.; Cheeke, P.R. 1993. Effects of feeding endophyte-infected tall fescue seed on lamb performance and serum prolactin. Proceedings of the 2nd International Symposium. on *Acremonium*/Grass Interactions. p. 111-113.

Delouis, C.; Djiane, J.; Houdebine, L.M.; Terque. 1980. Relation between hormones and mammary gland function. *Journal of Dairy Science* **63**: 1492-1513.

di Menna, M.E.; Lauren, D.R.; Sprosen, J.M.; MacLean, K.S. 1991. *Fusarium* and zearalenone on herbage fractions from short and long pasture. *New Zealand Journal of Agricultural Research* **34**: 445-452.

Donnelly, P.J.; Daily, R.A. 1991. Effects of dopamine, norepinephrine and serotonin on secretion of luteinizing hormone, follicle-stimulating hormone and prolactin in ovariectomized, pituitary stalk-transected ewes. *Domestic Animal Endocrinology* **8**: 87-98.

Doyle, A.E. 1988. The relevance of serotonin antagonism in the treatment of hypertension. *Drugs* **36**: 67-73.

Dunlap, S.E.; Vincent, C.K. 1971. Influence of postbreeding thermal stress on conception rate in beef cattle. *Journal of Animal Science* **32**: 1216.

Dyer, D.C. 1993. Evidence that ergovaline acts on serotonin receptors. *Life Science* **53**: 223-228.

Dyrmundsson, O.R. 1973. Puberty and early reproductive performance in sheep. II. Ram lambs. *Animal Breeding Abstracts* **41**: No.9, 419-430.

Easton, H.S.; Lane, G.A.; Tapper, B.A.; Keogh, R.G.; Cooper, B.M.; Blackwell, M.; Anderson, M.; Fletcher, L.R. 1996. Ryegrass endophyte-related heat stress in cattle. *Proceedings of the New Zealand Grasslands Association* **57**: 37-41.

Edwards, G.R.; Lucas, R.J.; Johnson, M.R. 1993. Grazing preference for pasture species by sheep is affected by endophyte and nitrogen fertility. *Proceedings of the New Zealand Grasslands Association* **55**: 137-141.

Eerens, J.P.J.; Easton, H.S.; Lucas, R.J.; White, G.H.; Miller, K.B. 1997. Influence of the ryegrass endophyte on sheep production in a cool-moist environment. Proceedings of the International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 413-416. Plenum Press, New York.

Eerens, J.P.J.; Lucas, R.J.; Easton, H.S.; White, J.G.H. 1998. Influence of the ryegrass endophyte (Neotyphodium lolii) in a cool moist environment. *New Zealand Journal of Agricultural Research* **41**: 39-48.

Eerens, J.P.J.; Miller, K.B.; White, J.G.H.; Easton, H.S.; Lucas, R.J. 1994. Ryegrass endophyte and sheep reproduction. *Proceedings of the New Zealand Grassland Association* **56**:255-258.

Ellis, K.J.; Zirkler, K.; Costigan, P. 1988. Confirmation of release rate from Captec chromium. *Controlled Release: Science and Technology Seminar* (Melbourne) 20-21.

Essig, H.W.; Cantrell, C.E.; Withers, F.T.; Lang, D.L.; Laughlin, D.H.; Boyd, M.E. 1989. Performance and profitability of cow-calf systems grazing on EF and EI KY-31 fescue (preliminary report). Proceedings of the Tall Fescue Toxicosis Workshop, Nov. 13-14, Atlanta. GA.

Evans, K.L.; Zavos, P.M.; Hemken, R.W.; Jackson, J.A. 1988. Effects of feeding endophyte-infected (*Acremonium coenophialum*) KY-31 fescue hay on reproductive development of Holstein bulls. *Theriogenology* **30**:169

Everts, H.; Alexander, G. (ed.); Barker, J.D. (ed); Slee, J. 1985. Relationships between the nutrition of the ewe, lamb birth weight and survival in prolific crossbreds. Factors affecting the survival of newborn lambs. A seminar in the CEC programme of co-ordination of agricultural research held in Brussels, 22 – 23 January, 1995. pp165-176.

Faichney, G.J.; Barry, T.N. 1986. Effects of mild heat exposure and suppression of prolactin secretion on gastro-intestinal tract function and temperature regulation in sheep. *Australian Journal of Biological Science* **39**: 85-97.

Fanning, M.D.; Spitzer, J.C.; Cross, D.L.; Thompson, F.N. 1992. A preliminary study of growth, serum prolactin and reproductive performance of beef heifers grazing *Acremonium coenophialum*-infected tall fescue. *Theriogenology* **38**: 375-384.

Fletcher, L.R. personal communication. AgResearch Lincoln Research Centre, Lincoln, New Zealand.

Fletcher, L.R. 1993. Heat stress in lambs grazing ryegrass with different endophytes. Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. Hume, Latch and Easton (Eds). p 114-118.

Fletcher, L.R.; Barrell, G.K. 1984. Reduced liveweight gains and serum prolactin levels in hoggets grazing ryegrass containing Lolium endophyte. *New Zealand Veterinary Journal* **32**: 139-140.

Fletcher, L.R.; Easton, H.S. 1997. The evaluation and use of endophytes for pasture improvement. Proceedings of the International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 209-227. Plenum Press, New York.

Fletcher, L.R.; Harvey, I.C. 1981. An association of *Lolium* endophyte with ryegrass staggers. *New Zealand Veterinary Journal* **29**: 185-186.

Fletcher, L.R.; Sutherland, B.L. 1993. Flystrike and faecal contamination in lambs grazing endophyte infected ryegrasses. Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. Hume, Latch and Easton (Eds). p 122-124.

Fletcher, L.R.; Sutherland, B.L.; Fletcher, C.G. 1997. Effect of ambient and black-globe temperature on plasma prolactin levels in ewes grazing endophyte-free and endophyte infected ryegrass. Proceedings of the 3rd International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 425-428. Plenum Press, New York.

Fletcher, L.R.; Sutherland, B.L.; Fletcher, C.G. 1999. The impact of endophyte on the health and productivity of sheep grazing ryegrass-based pastures. *Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series No. 7*: 11-18.

Fletcher, L.R.; Sutherland, B.L. ;Fletcher, C.G.; Easton, H.S. 1996. The impact of endophyte toxins on the health of grazing sheep - an evolving story. Proceedings of the Second Pan Pacific Veterinary Conference Sheep. 31-42.

Flint, D.J.; Clegg, R. A.; Vernon, R.G. 1981. Prolactin and the regulation of adipose-tissue metabolism during lactation in rats. *Molecular and Cellular Endocrinology* **22**: 2, 265-275.

Flux, D.S.; Mackenzie, D.D.S.; Wilson, G.F. 1984. Plasma metabolite and hormone concentrations in Friesian cows of differing genetic merit measured at two feeding levels. *Animal Production* **38**: 377-384.

Forsyth, I.A. 1986. Variation among species in the endocrine control of mammary growth and function: the roles of prolactin, growth hormone and placental lactogen. *Journal of Dairy Science* **69**: 886-903.

Forsyth, I.A.; Lee, P.D. 1993. Bromocriptine treatment of periparturient goats: long-term suppression of prolactin and lack of effect on lactation. *Journal of Dairy Research* **60**: 307-317.

Fowler, D.G.; Kennedy, J.P. 1967. Skin folds and Merino breeding: The effects of varying heat exposures and degree of skin fold on rectal, scrotal and testis temperatures. *Australian Journal of Experimental Agriculture and Animal Husbandry* **8**: 133-141.

Gadberry, M.S.; Denard, T.M.; Spiers, D.E.; Piper, E.L. 1997. Ovis aries: A model for studying the effects of fescue toxins on animal performance in a heat-stress environment. Proceedings of the 3rd International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 429-431. Plenum Press, New York.

Gallagher, R.T.; Keogh, R.G.; Latch, G.C.M.; Reid, C.S.W. 1977. The role of fungal tremorgens in ryegrass staggers. *New Zealand Journal of Agricultural Research* **20**: 431-440.

Ganong, W.F. (Ed). 1989. Review of medical physiology (14th Ed). Appleton and Lang.

Garrett, L.W.; Heimann, E.D.; Pfander, W.H.; Wilson, L.L. 1980. Reproductive problems of pregnant mares grazing fescue pastures. *Journal of Animal Science* **51**(Suppl. 1):237 (Abstr.).

Gay, N.; Boling, J.A.; Dew, R.; Miksch, D.E. 1988. Effects of endophyte-infected tall fescue on beef cow-calf performance. *Applied Agricultural Research* **3**:182.

Gaynor, D.L.; Rowan, D.D.; Chapman, R.B. (ed.) 1985. Peramine - an Argentine stem weevil feeding deterrent from endophyte-infected ryegrass. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology, held at Lincoln College, University College of Agriculture, Canterbury, New Zealand, 13-17 May, 1985. 338-343.

Geenty, K.G.; Clarke, J.N.; Wright, D.E. 1985. Lactation performance, growth, and carcass composition of sheep. 2. Relationship between ewe milk production, lamb water turnover, and lamb growth in Romney, Dorset, and crossbred sheep. *New Zealand Journal of Agricultural Research* **28**: 248-255.

Geenty, K.G.; Dyson, C.B. 1986. The effects of various factors on the relationship between lamb growth rate and ewe milk production. *Proceedings of the New Zealand Society of Animal Production* **46**: 265-269.

Genicot, B.; Mouligneau, F.; Lindsey, J.K.; Lambert, P.; Close, R.; Lekeux, P. 1993. Induction of a serotonin-2 receptor blockade during early or late stage of acute respiratory distress syndrome in double-muscled calves: a comparative study. *Journal of Veterinary Medicine* **40**: 241-248.

Georke, T.P. 1974. Embryo survival in ewes heat stressed during early placentogenesis. *Dissertation Abstracts International,-B* **35**: 9, 4302-4303.

Gibb, M.J.; Treacher, T.T. 1978. The effect of herbage allowance of herbage intake and performance of ewes and their twin lambs grazing perennial ryegrass. *Journal of Agricultural Science* **90**: 1, 139-147.

Gibb, M.J.; Treacher, T.T. 1982. The performance of lactating ewes and weaned lambs offered diets containing different proportions of perennial ryegrass and white clover. *Animal Production* **34**: 403.

Goldsmith, P.C.; Cronin, M.J.; Weiner, R.I. 1979. Dopamine receptor sites in the anterior pituitary. *Histochemistry and. Cytochemistry* **27**: 1205-1207.

Gonzales, G.F.; Coyotupa, J.; Guerra-Garcia, R. 1981. Effects of serotonin and its precursor on the testicular function in male rats. *IRCS Medical Science: Library Compendium* **9**: 61.

Gow, C.B.; McDowell, G.H.; Jenkin, G. 1983. The importance of prolactin for initiation of lactation in the pregnant ewe. *Australian Journal of Biological Science* **36**: 357-367.

Gwazdauskas, F.C.; Thatcher, W.W.; Kiddy, C.A.; Paape, M.J.; Wilcox, C.J. 1981. Hormonal patterns during heat stress following PGF2 alpha -tham salt induced luteal regression in heifers. *Theriogenology* **16**: 271-285.

Hannah, S.M.; Paterson, J.A.; Williams, J.E.; Kerley, M.S.; Miner, J.L. 1990. Effects of increasing dietary levels of endophyte-infected tall fescue seed on diet digestibility and ruminal kinetics in sheep. *Journal of Animal Science* **68**: 1693-1701.

Harmon, D.L.; Gross, K.L.; Kreikemeier, K.K.; Coffey, K.P.; Avery, T.B.; Klindt, J. 1991. Effects of feeding endophyte-infected fescue hay on portal and hepatic nutrient flux in steers. *Journal of Animal Science* **69**: 1223-1231.

Harper, F.; Henton, J.1981. Reproductive problems in pregnant broodmares associated with fescue forage. University Tennessee Extension.

Harris, W.; Hoglund, J.H. 1980. Influences of seasonal growth periodicy and N fixation on competitive combining abilities of grasses and legumes. *Proceedings XIII International Grassland Congress*: 239-243.

Hart, I.C. 1976. Prolactin, growth hormone, insulin and thyroxine: their possible roles in steroid-induced mammary growth and lactation in the goat. *Journal of Endocrinology* **71**: 41-42.

Hart, I.C.; Morant, S.V. 1980. Roles of prolactin, growth hormone, insulin and thyroxine in steroid-induced lactation in goats. *Journal of Endocrinology* **84**: 343-351.

Hartley, M.J.; Blank, R.H.; Bell, D.S.; Page, C.R.; Olson, M.H.; M.J. Hartley (ed.). 1982.Cricket damage on the Kaipara clay flats Ruawai in 1982. Proceedings of the thirty-fifth New Zealand Weed and Pest Control Conference. Waikato Motor Hotel, August 9th to 12th, 1982. 113-118.

Hartmann, P.E.; Trevethan, P.; Shelton, J.N. 1973. Progesterone and oestrogen and the initiation of lactation in ewes. *Journal of Endocrinology* **59**: 249-259.

Hawker, H. 1977. Effect of age on sheep production in an arid environment. *Dissertation Abstracts International, B* **38**: 1, 2.

Hemken, R.W.; Bull, L.S.; Boling, J.A.; Kane, E.; Bush, L.P.; Buckner, R.C. 1979. Summer fescue toxicosis in lactating dairy cows and sheep fed experimental strains of ryegrass-tall fescue hybrids. *Journal of Animal Science* **49**: 641.

Hemken, R.W.; Boling, J.A.; Bull, L.S.; Hatton, R.H.; Buckner, R.C.; Bush, L.P. 1981. Interaction of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. *Journal of Animal Science* **52**: 710.

Hill, T.G. 1980. Effects of induced hyperthermia on plasma concentrations of luteinizing hormone, prolactin, progesterone and testosterone in sheep. *Dissertation Abstracts International* **41**: 87.

Hill, T.G.; Alliston, C.W. 1981. Effects of thermal stress on plasma concentrations of luteinizing hormone, progesterone, prolactin and testosterone in the cycling ewe. *Theriogenology* **15**: 201-209.

Hooley, R.D.; Campbell, J.J.; Findlay, J.K. 1978. The importance of prolactin for lactation in the ewe. *Journal of endocrinology* **79**: 301-310.

Hopkins, P.S.; Nolan, C.J.; Pepper, P.M. 1980. Effects of heat stress on the development of the foetal lamb. *Australian Journal of Agricultural Research* **4**: 763-771.

Hoveland, C.S. 1992. Importance and economic significance of *Acremoniun* endophytes to performance of animals and grass plants. In: R.E. Joost and S.S. Quisenberry (Ed.) Proceedings of the 1st International Symposium On *Acremonium*/grass interactions. November 5-7, 1990. Elsevier Scientific Publishers, Amsterdam, The Netherlands.

Hoveland, C.S.; Haaland, R.L.; King, C.C.; Anthony, W.B.; Clark, E.M.; McGurire, J.A.; Smith, L.A.; Grimes, H.W.; Holliman, J.L. 1983. Steer performance and association of *Acremonium coenophialum* fungal endophyte on tall fescue pasture. *Agronomy Journal* **75**:821.

Hoveland, C.S.; Haaland, R.L.; King, C.C.; Anthony, W.B.; Clark, E.M.; McGurire, J.A.; Smith, L.A.; Grimes, H.W.; Holliman, J.L. 1980. Association of *Epichloe typhina* fungus and steer performance on tall fescue pastures. *Agronomy Journal* **72:**1064.

Howard, M.D.; Muntifering, R.B.; Bradley, N.W.; Mitchell, G.E.; Lowry, S.R. 1992. Voluntary intake and ingestive behaviour of steers grazing Johnstone or endophyte-infected Kentucky-31 tall fescue. *Journal of Animal Science* **70**: 1227.

Howles, C.M.; Webster, G.M.; Haynes, N.B. 1980. The effect of rearing under a long or short photoperiod on testis growth, plasma testosterone and prolactin concentrations, and the development of sexual behaviour in rams. *Journal of Reproduction and Fertility* **60**: 437-447.

Hume, D.E.; Popay, A.J.; Barker, D.J. 1993. Effect of *Acremonium* endophyte on growth of ryegrass and tall fescue under varying levels of soil moisture and argentine stem weevil attack. Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. Hume, Latch and Easton (Eds). p 161-164.

Jackson, J.A.; Hemken, R.W.; Boling, J.A.; Buckner, R.C. 1988. Effect of feeding *Acremonium coenophialum* infected tall fescue to primiparous dairy heifers and feeding an *Acremonium* free selection to dairy calves on the expression of fescue toxicosis. *Nutrition Reports International* **37**: 1265-1274.

Jamieson, W.S.; Hodgson, J. 1979. The effect of variation in sward characteristics upon the ingestive behaviour and herbage intake of calves and lambs under continuous stocking management. *Grass and Forage Science* **34**: 273-282.

Johnsson, I.D.; Hart, I.C. 1985. Pre-pubertal mammogenesis in the sheep. 1. The effects of level of nutrition on growth and mammary development in female lambs. *Animal Production* **41**: 323-332.

Johnsson, I.D.; Hart, I.C.; Turvey, A. 1986. Pre-pubertal mammogenesis in sheep. 3. The effects of restricted feeding or daily administration of bovine growth hormone and bromocriptine on mammary growth and morphology. *Animal Production* **42**: 53-63.

Judd, S.J.; Rakoff, J.S.; Yen, S.S.C. 1978. Inhibition of gonadotropin and prolactin release by dopamine: Effect of endogenous estradiol levels. *Journal of Clinical Endocrinology and Metabolism* **47**: 494-498.

Kann, G. 1976a. Inhibition of prolactin secretion in the ewe by 2-Br-ergocriptine during pregnancy or early lactation: effect on milk yield. Abstracts of short communications. 5th International Congress of Endocrinology July 18-24 Hamburg. 251.

Kann, G. 1976b. Influence of the suppression of the prepartum surge of prolactin by ergocryptine on the milk yield and on the postpartum anoestrous of the nursing ewe. *Annales de biologie, animal biochimie, biophysique* **16**: 163.

Kann, O. 1971.Variations des concentrations plasmatiques de l'hormone luteinisante et de la prolactine au cours du cycle oestrien de la brebis. [Variations in the plasma concentrations of luteinizing hormone and prolatin during the oestrous cycle of the ewe]. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences* **272d**: No.23, 2934-2937.

Kann, G.; Carpentier, M.C.; Fevre, J.; Martinet, J.; Maubon, M.; Meunier, C.; Play, J.; Vermiere, N. 1977. Lactation and prolactin in sheep, role of prolactin in initiation of milk secretion. In: *Progress in Prolactin Physiology and Pathology*. Rabyn, C. & Harter, M. (Eds). Elsevier/Noth-Holland Biomedical Press, Amsterdam 1978. pp. 201-212.

Kann, G.; Denamur, R. 1974. Possible role of prolactin during the oestrous cycle and gestation in the ewe. *Journal of Reproduction and Fertility* **39**: 473-483.

Karg, H.; Schams, D. 1974. Prolactin release in cattle. *Journal of Reproduction and Fertility* **39**: 463.

Keane, M.G. 1974. Effect of bodyweight on attainment of puberty and reproductive performance in Suffolk X ewe lambs. *Irish Journal of Agricultural Research* **13**: 3, 263-274.

Keogh, R.G. 1973. Induction and prevention of ryegrass staggers in grazing sheep. *New Zealand Journal of Experimental Agriculture* **1**: 55-57.

Keogh, R.G.; Blackwell, M.B.; Shepherd, P. 1999. Performance of dairy cows grazing pastures with or without ergovaline and lolitrem B in Northland. *Proceedings of the New Zealand Society of Animal Production* **59**: 254-257.

Keogh, R.G.; Tapper, B.A.; Fletcher, R.H. 1996. Distributions of the fungal endophyte *Acremonium lolii*, and of the alkaloids lolitrem B and peramine, within perennial ryegrass. *New Zealand Journal of Agricultural Research* **39**: 121-127.

Knight, T.N. 1990. Reproductive wastage, a guide for fundamental research: A New Zealand perspective. *In* Reproductive Pysiology of the Merino Sheep. Oldham, G.M.; Martin, G.R. and Purvis, I.W. (Eds).

Kramer, R. 1997. Zearalenone in pasture and its effects on reproduction in ewes. MapplSc. thesis, Massey University, Palmerston North, New Zealand, 76pp.

Kramer, R.; Keogh, R.G.; McDonald, M.F. 1999. Effects of ergovaline in endophyte-infected tall fescue on ewe fertility. *Proceedings of the New Zealand Society of Animal Production* **59**: 263-265.

Krisch, I.; Budihna, M.V.; Rucman, R. 1992. Structure-activity study of some newly synthesised ergoline derivatives on 5-HT_2 receptors and alpha-adrenoceptors in rabbit isolated aorta. *Pharmacology* **45**: 195-208.

Lamberts, S.W.J.; Macleod, R.M. 1990. Regulation of prolactin secretion at the level of the lactotroph. *Physiology Review* **70**:279.

Lancashire, J.A. 1984. The distribution and use of forage legumes in New Zealand. In: *Forage Legumes for Energy-Efficient Animal Production - Proceedings of a Trilateral Workshop, Palmerston North, New Zealand*: 20-33.

Lane, G.A.; Tapper, B.A.; Davies, E. Christensen, M.J.; Latch, G.C.M. 1997b. Occurrence of extreme alkaloid levels in endophyte-infected perennial ryegrass, tall fescue and meadow fescue. Proceedings of the 3rd International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 433-436. Plenum Press, New York.

Lane, G.A.; Tapper, B.A.; Davies, E. Hume, D.E.; Latch, G.C.M.; Barker, D.J.; Easton, H.S.; Rolston, M.P. 1997a. Effect of growth conditions on alkaloid concentrations in perennial ryegrass naturally infected with endophyte. Proceedings of the 3rd International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 311-346. Plenum Press, New York.

Le Neindre, P.; Poindron, P.; Delouis, C. 1979. Hormonal induction of maternal behaviour in non-pregnant ewes. *Physiology and Behaviour* **22**: 731-734.

Linsay, D.; Poulson, E.; Robson, J.M. 1963. The effect of 5-hydroxytryptamine on pregnancy. *Journal of Endocrinology* **26**: 85-96.

Lopez, G.C.; Ulloa, A.R.; Rochinn, S. 1990. Factors affecting the weight of lamb per ewe at birth in Corriedale sheep. *Memoria – III Congreso Nacional de Produccion Ovina.* 47-49.

Louw, B.P.; Lishman, A.W.; Botha, W.A.; Baumgartner, J.P. 1974. Failure to demonstrate a role for the acute release of prolactin at oestrus in the ewe. *Journal of Reproduction and Fertility* **40**: 455-458.

McCann, J.S.; Heusner, G.L.; Amos, H.E.; Tompson, D.L. 1992. Growth rate, diet digestibility, and serum prolactin of yearling horses fed non-infected and infected tall fescue hay. *Journal of Equine Veterinary Science* **12**: 240-243.

McDonald, W.T. 1989. Performance of cows and calves grazing endophyte-infested pasture. M.S. Thesis. University of Tennessee, Knoxville.

Mackintosh, C.G.; Orr, M.B.; Gallagher, R.T.; Harvey, I.C. 1982. Ryegrass staggers in Canadian Wapiti deer. *New Zealand Veterinary Journal* **30**: 106-107.

McKenzie, F.F.; Berliner, V. 1937. The reproductive capacity of rams. University of Missouri, Agricultural Experiment Station, Research Bulletin No. 265.

Mahendra-Singh; Ludri, R.S.; Singh, M. 1999. Immediate effect of bromocryptine on plasma hormone concentrations during early lactation in crossbred goats. *Small Ruminant Research* **31**: 141-147.

Mahmood, T.; Ott, R.S.; Foley, G.L.; Zinn, G.M.; Schaeffer, D.J.; Kesler, D.J. 1994. Growth and ovarian function of weanling and yearling beef heifers grazing endophyte-infected tall fescue pastures. *Theriogenology* **42**: 1149-1158.

Mainoya, J.R. 1975. Analysis of the role of endogenous prolactin on fluid and sodium chloride absorption by the rat jejunum. *Journal of Endocrinology* **67**: 343-349.

Mainoya, J.R. 1981. Colon absorption of water and NaCl in the rat during lactation and the possible involvement of prolactin. *Experientia* **37**: 1083-1084.

Mantle, P.G. 1983. Amino acid neurotransmitter release from cerebrocortical synaptosomes of sheep with severe ryegrass staggers in New Zealand. *Research in Veterinary Science* 34: 3, 373-375.

Martin, G.R. 1994. Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. *Pharmacology and Therapy* **62**: 283-324.

Mellor, D.J.; Flint, D.J.; Vernon, R.G.; Forsyth, I.A. 1987. Relationships between plasma hormone concentrations, udder development and the production of early mammary secretions in twin-bearing ewes on different planes of nutrition. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **72**: 345-356.

Milne, J.A.; Hodgson, J.; Thompson, R.; Souter, W.G.; Barthram, G.T. 1982. The diet ingested by sheep grazing swards differing in white clover and perennial ryegrass content. *Grass and Forage Science* **37**: 209-218.

Mitchell, J.A.; Hammer, R.E. 1983. Serotonin-induced disruption of implantation in the rat: I. Serum progesterone, implantation site blood flow, and intrauterine pO2. *Biology of Reproduction* **28**: 830-835.

Mitchell, P.J.; McCaughan, C.J. 1992. Perennial ryegrass staggers in fallow deer (*Dama dama*). *Australian Veterinary Journal* **69**: 258-259.

Mizinga, K.M.; Thompson, J.A.; Stuedemann, J.A.; Kiser, T.E. 1992. Effects of feeding diets containing endophyte-infected fescue seed on luteinizing hormone secretion in postpartum beef cows and cyclic heifers and cows. *Journal of Animal Science* **70**: 3483.

Monroe, J.L.; Cross, D.L.; Hudson, L.W.; Hendricks, D.M.; Kennedy, S.W.; Bridges, W.C. 1988. Effects of selenium and endophyte-contaminated fescue on the performance and reproduction in mares. *Equine Veterinary Science* **8**: 148.

Monty, D.E.; Racowsky, C. 1987. In vitro evaluation of early embryo viability and development in summer heat-stressed, superovulated dairy cows. *Theriogenology* **28**: 451.

Moore, C.R.; Oslund, R. 1924. Experiments on the sheep testis-cryptorchidism, vasectomy, and scrotal insulation. *American Journal of Physiology* **67**: 595.

Morley, F.W.H.; White, D.H.; Kenney, P.A.; Davis, I.F. 1978. Predicting ovulation rate from liveweight in ewes. *Agricultural Systems* **3**: 1, 27-45.

Munday-Finch, S.C.; Garthwaite, I. 1999. Toxicology of ryegrass endophyte in livestock. *Ryegrass Endophyte: An Essential New Zealand Symbiosis. Grassland Research and Practice Series* No. 7: 63-68.

Munro, C.J.; McNatty, K.P.; Renshaw, L. 1980. Circa-annual rhythms of prolactin secretion in ewes and the effect of pinealectomy. *Journal of Endocrinology* **84**: 83-89.

National Institute of Water and Atmospheric Research. Mean daily air temperatures 1969-1998. *http://www.metservice.co.nz./knowledge/data_mean_min_air_temps.asp*

Neal, W.D.; Schmidt, S.P. 1985. Effects of feeding Kentucky 31 tall fescue seed infected with Acremonium coenophialum to laboratory rats. *Journal of Animal Science* **61**: 603-611.

Neill, J.C. 1941. The endophytes of *Lolium* and *Festuca. New Zealand Journal of Science and Technology* **23**: 185-193.

Nequir, L.G.; King, S.S.; Johnson, A.L.; Gow, G.M.; Ferreira-Dias, G.M. 1993. Prolactin may play a role in stimulating the equine ovary during the spring reproductive transition. *Journal of Equine Veterinary Science* **13**: 11, 631-635.

Nicoll, C.S.; Bryant, G.D. 1972. Physiological and immunological properties of prolactin. Proceedings of the 4th Tenovus Workshop: Prolactin and Carcinogenesis. P. 28.

Nolan, A.; Livingston, A.; Waterman, A. 1986. The effects of alpha$_2$ adrenoreceptor agonists on airway pressure in anesthetized sheep. *Journal of Veterinary Pharmacology and Therapy* **9**: 157-163.

Numan, M.; Rosenblatt, J.S.; Komisaruk, B.R. 1977. Medial preoptic area and onset of maternal behaviour in the rat. *Journal of Comparative Physiology and Psychology* **91**: 146-167.

Oliver, J.W. 1997. Physiological manifestations of endophyte toxicosis. Proceedings of the 3rd International Symposium on *Neotyphodium*/Grass Interactions. Bacon C.W. and Hill, N.S. (Eds). p. 311-346. Plenum Press, New York.

Oliver, J.W.; Powell, R.G.; Abney, L.K.; Linnabary, R.D.; Petroski, R.J. 1990. N-acetyl loline-induced vasoconstriction of the lateral saphenous vein (cranial branch) of cattle. Proceedings of the 1st International Symposium on *Acremonium*/Grass Interactions. Quisenberry, S.S. and Joost, R.E. (Eds). p. 239-243.

Oliver, J.W.; Abney, L.K.; Strickland, J.R.; Linnabary, R.D. 1993a. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. *Journal of Animal Science* **71**: 2708-2713.

Oliver, J.W.; Linnabary, R.D.; Abney, L.K.; Strickland, J.R. 1993b. Response of blood vessels to N-acetyl loline and serotonin. *Proceedings of the Tall Fescue Toxicosis Workshop*, SERAIEG-8, Atlanta, GA. Oct. 25-26. Pg. 51.

Oliver, J.W.; Linnabary, R.D.; Strickland, J.R.; Schultze, A.E.; Waller, J.C.; Fribourg, H.A.; Abney, L.K.; Bailey, E.M.; Barnhill, M.A. 1996. Characterisation of inflammatory response to ergot alkaloid presence in toxic tall fescue. Unpublished data.

Orr, M.B.; Mackintosh, C.G. 1985. Ryegrass staggers in deer. Proceedings of a deer course for veterinarians, Ashburton, New Zealand, July 1985, 39-43 Veterinary Association, New Zealand.

Osborn, T.G.; Schmidt, S.P.; Marple, D.N.; Rahe, C.H.; Steenstra, J.R. 1992. Effect of consuming fungus-infected tall fescue and ergotamine tartarate on selected physiological variables of cattle in environmentally controlled conditions. *Journal of Animal Science* **70**: 2501-2509.

Parker, W.J.; McCutcheon, S.N.; Carr, D.H. 1989. Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. *New Zealand Journal of Agricultural Research* **32**: 537-546.

Parker, W.J.; Morris, S.T.; Garrick, D.J.; Vincent, G.L.; McCutcheon, S.N. 1990. Intraruminal chromium controlled release capsule for measuring herbage intake in ruminants- a review. *Proceedings of the New Zealand Society of Animal Production* **50**: 437-442.

Pashen, R.L. 1984. Maternal and foetal endocrinology during late pregnancy and parturition in mares. *Equine Veterinary Journal* **16**: 233-238.

Paterson, J.; Forcherio, C.; Larson, B.; Samford, M.; Kerley, M. 1995. The effects of fescue toxicosis of beef cattle productivity. *Journal of Animal Science* **73**: 889-898.

Peterson, S.W. 1992. The role of prolactin in the control of ovine lactogenesis. PhD thesis, Massey University, Palmerson North, New Zealand. 158pp.

Peterson. S.W.; Mackenzie, D.D.S.; McCutcheon, S.N 1990. Milk production and plasma prolactin levels in spring- and autumn-lambing ewes. *Proceedings of the New Zealand Society of Animal Production* **50**:483-485.

Peterson. S.W.; Mackenzie, D.D.S.; McCutcheon, S.N.; Lapwood, K.R. 1997. Long-term bromocriptine treatment during late pregnancy has differential effects on milk yields of single- and twin-bearing ewes. *New Zealand Journal of Agricultural Research* **40**: 249-2569.

Philo, R.; Reiter, R.J. 1980. A circannual rhythm in bovine pineal serotonin. *Experientia* **36**: 664-665.

Polkowska, J.; Wolinska, E.; Domanski, E. 1976. Cyclic activity of the pituitary prolactin cells and plasma prolactin levels in the oestrous cycle of the ewe. *Journal of Reproduction and Fertility* **46**: 295-298.

Poppenga, R.H.; Monstrom, M.S.; Haschek, W.H.; Lock, T.F.; Buck, W.B.; Beasely, V.R. 1984. Mare agalactia, placental thickening and high mortality associated with the grazing of tall fescue : a case report. p 326. *American Association of Veterinary Laboratory Diagnostics* 27th Annual Proceedings.

Porter, J.K.; Thompson, F.N. 1992. Effects of fescue toxicosis on reproduction in livestock. *Journal of Animal Science* **70**:1594-1603.

Porter, J.K.; Robbins, J.D.; Norred, W.P.; Garner, G.B.; Bacon, C.W. 1985. Serum prolactin and brain catecholamine metabolite depression in rats administered extracts of endophyte-infected fescue. *Journal of Agricultural and Food Chemistry* **33**:34-360.

Pownall, D.B. 1992. Scouring and high endophyte ryegrass. "Modernising the family farm". Organised by Lincoln University Farmers Committee, published by the Centre for Continuing Education, Lincoln University.

Pownall, D.B.; Lucas, R.J.; Familton, A.S.; Love, B.G.; Fletcher, L.R. 1993. Endophyte associated mycotoxins and diarrhoea in lambs. Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. Hume, Latch and Easton (Eds). p 132-134.

Prestidge, R.A.; Ball O.J.-P. 1996. A Catch-22 on the utilisation of endophytic fungi for pest management. In "*Multitrophic Interactions in Terrestrial Ecosystems*"" Gange, A.D, and Brown, V.K. (eds). Blackwell Scientific Press, London.

Prestidge, R.A.; di Menna, M.E.; van der Zijpp, S.; Badan, D. 1985. Ryegrass content, *Acremonium* endophyte and Argentine stem weevil in pastures in the volcanic Plateau. Proceedings 38th New Zealand Weed and Pest Control Conference: 41-44.

Prestidge, R.A.; Popay, A.J.; Ball, O.J-P. 1994. Biological control of pastoral pests using *Acremonium* spp. endophytes. *Proceedings of the New Zealand Grassland Association* **56**: 33-38.

Prestidge, R.A.; Thom, E.R.; Marshall, S.L.; Taylor, M.J.; Willoughby, B.; Wildermoth, D.D. 1992. Influence of *Acremonium lolii* infection in perennial ryegrass on germination, emergence, survival, and growth of white clover. *New Zealand Journal of Agricultural Research* **35**: 225-234.

Putnam, M.R.; Bransby, D.I.; Schumacher, J.; Boosinger, T.R.; Bush, L.; Shelby, R.A.; Vaughan, J,T; Ball, D.; Brendemuehl, J.P. 1991. Effects of fungal endophyte *Acremonium coenophialum* in fescue on pregnant mares and foal viability. *American Journal of Veterinary Research* **52**:2071.

Quigley, P.; Li, X.; McDonald, G.; Noske, A. 1993. Effects of *Acremonium lolii* on mixed pastures and associated insect pests in south-eastern Australia. Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. Hume, Latch and Easton (Eds). p 177-179.

Quinlivan, T.D.; Martin, C.A. 1971. Survey observations on the reproductive performance of both Romney stud and commercial flocks throughout New Zealand. II. Lambing data from an intensive survey in stud flocks. III. National commercial flock performance. *New Zealand Journal of Agricultural Research* **14**: 858-879.

Ravault, J.P.; Courot, M.; Garnier, D.; Pelletier, J.; Terqui, M. 1977. Effect of 2-Bromo-α-Ergocryptine (CB 154) on plasma prolactin, LH and testosterone levels, accessory reproductive glands and spermatogenesis in lambs during puberty. *Biology of Reproduction* **17**:192-197.

Redmond, L.M.; Cross, D.L.; Jenkins, T.C.; Kennedy, S.W. 1991. The effect of *Acremonium coenophialum* on intake and digestibility of tall fescue hay in horses. *Journal of Equine Veterinary Science* **11**: 215-219.

Redmond, L.M.; Cross, D.L.; Kennedy, S.W. 1993. Effect of three levels of domperidone on gravid mares grazing endophyte (*Acremonium coenophialum*) infected tall fescue. *Journal of Animal Science* **71**(Suppl 1): 16 (Abstr.)

Redmond, L.M.; Cross, D.L.; Strickland, J.R.; Kennedy, S.W. 1994. Efficacy of domperidone and sulpiride for fescue toxicosis in horses. *American Journal of Veterinary Research* **55**:722-729.

Rhind, S.M,; Robinson, J.J.; Chesworth, J.M.; Crofts, R.M.J. 1980. Effects of season, lactation and plane of nutrition on prolactin concentrations in ovine plasma and the role of prolactin in the control of ewe fertility. *Journal of Reproduction and Fertility* **58**, 1: 145-152.

Rhodes, M.T.; Aldrich, C.G.; Paterson, J.A.; Kerley, M.S. 1989. The effect of endophyte-infected tall fescue and a dopamine antagonist on relative blood flow in sheep. *Journal of Animal Science* **67** (Suppl. 1):286 (Abstr.).

Rhodes, M.T.; Paterson, J.A.; Kerley, M.S.; Garner, H.E.; Laughlin, M.H. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. *Journal of Animal Science* **69**: 2033-2043.

Rhodes, R.C.; Randel, R.D. 1982. Effect of several biogenic amines on in vitro progesterone secretion by the bovine corpus luteum. *Comparative Biochemistry Physiology* **72**: 113-116.

Rice, R.L.; Schurig, G.G.; Blodgett, D.J.; Swecker, W.S.; Fontenot, J.P.; Allen, V.G.; Akers, R.M. 1995. Humoral immune responses of cattle maintained of fescue pastures. *Proceedings of the Tall Fescue Toxicosis Workshop*, SERAIEG-8, Nashville, TN.p. 69-70.

Rodway, R.G.; Robinson, J.J.; Phillippo, M. 1983. Ovulation rate in induced oestrous cycles of anoestrous ewes given bromocriptine. *Journal of Reproduction and Fertility* **68**: 265-265.

Rowan, D.D.; Shaw, G.J. 1987. Detection of ergopeptine alkaloids in endophyte-infected perennial ryegrass by tandem mass spectrometry. *New Zealand Veterinary Journal* **35**: 197-198.

Rowan, D.D.; Tapper, B.A.; Sergejew, N.L.; Latch, G.C.M. 1990. Ergopeptine alkaloids in endophyte-infected ryegrass and fescues in New Zealand. *Proceedings of the 1st international symposium on Acremonium/grass interactions* Ed R. Joost & S. Quisenberry. Louisiana Agricultural Experimental Station. pp 97-99.

Ruckebusch, Y.; Ooms, L. 1983. Selective blockade of the responses of reticulo-ruminal muscle to 5-HT in sheep. *Journal of Veterinary Pharmacology and Therapy* **6**: 127-132.

Salah, M.S.; Al-Shaikh, M.A.; Al-Saiady, M.Y.; Mogawer, H.H. 1995. Effect of prolactin inhibition on thermoregulation, water and food intakes in heat-stressed fat-tailed male lambs. *Animal Science* **60**: 87-91.

Samford-Grigsby, M.D.; Larson, B.T.; Forcherio, J.C. 1997. Injection of a dopamine antagonist into Holstein steers to relieve symptoms of fescue toxicosis. *Journal of Animal Science* **75**: 1026-1031.

Sasaki, Y.; Takahashi, H. 1980. Insulin secretion in sheep exposed to cold. *Journal of Physiology* **306**: 323-335.

Sasaki, Y.; Takahashi, H.; Aso, H.; Ohneda, A.; Weekes, T.E.C. 1982. Effects of cold exposure on insulin and glucagon secretion in sheep. *Endocrinology* **111**: 2070-2076.

Scales, G.H.; Burton, R.N.; Moss, R.A. 1986. Lamb mortality, birthweight, and nutrition in late pregnancy. *New Zealand Journal of Agricultural Research* **29**: 1, 75-82.

Schams, D.; Reihardt, V.; Karg, H. 1972. Effects of 2-BR-alpha-ergokryptine on plasma prolactin level during parturition and onset of lactation in cows. *Experientia* **28**: 687.

Schams, D.; Russe, I.; Schallenberger, E.; Prokopp, S.; Chan, J.S.D. 1984. The role of steroid hormones, prolactin and placental lactogen on mammary gland development in ewes and heifers. *Journal of Endocrinology* **102**: 121-130.

Schmidt, S.P.; Danilson, D.A.; Holliman, J.A.; Grimes, H.W.; Webster, W.B. 1986. Fescue fungus suppresses growth and reproduction in replacement beef heifers. Highlights of Agricultural Research 33:15 Alabama Agricultural Experimental Station, Auburn Univ., Auburn.

Sharp, D.C.; Bozar, F.W. 1995. Equine reproduction VI. *Biology of Reproduction* Monograph Series 1.

Shennan, D.B. 1994. Regulation of water and solute transport across mammalian plasma cell membranes by prolactin. *Journal of Dairy Research* **61**: 155-166.

Siegel, G.; Agranoff, B.; Albers, R.W.; Molinoff, P. 1989. Basic Neurochemistry (4th Ed.). Raven Press, New York.

Siegel, H.I.; Rosenblatt, J.S. 1975. Progesterone inhibition of oestrogen induced maternal behaviour in hysterectomized ovariectomized virgin rats. *Hormones and Behaviour* **6**: 223-230.

Slotin, C.A.; Harrison, F.A.; Heap, R.B. 1971. Kinetics of progesterone metabolism in the pregnant sheep. *Journal of Endocrinology* **49**: 30-69.

Smeaton, D.C.; Hockey, H.U.P.; Towers, N.R. 1985. Effects of facial eczema on ewe reproduction and ewe and lamb live weights. *Proceedings of the New Zealand Society of Animal Production* **45**: 133-135.

Smith, S.M. 1980. Role of prolactin in regulating gonadotrophin secretion and gonad function in female rats. *Federal Proceedings* **39**:2571.

Smith, B.L.; McLeay, L.M.; Embling, P.P. 1997. Effect of the mycotoxins penitrem, paxilline and lolitrem B on the electromyographic activity of skeletal and gastrointestinal smooth muscle of sheep. *Research in Veterinary Science* **62**: 111-116.

Stamm, M.M.; DelCurto, T.; Horney, M.R.; Brandyberry, S.D.; Barton, R.K. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. *Journal of Animal Science* **72**: 1068-1075.

Stevens, D.R.; Drew, K.;Laas, F.; Turner, J.D. 1992. Deer production from ryegrass- and tall fescue-based pastures. *Proceedings of the New Zealand Grassland Association* **54**: 23-26.

Stilham, W.D.; Brown, C.J.; Daniels, L.B.; Piper, E.L.; Fetherstone, H.E. 1982. Toxic fescue linked to reduced milk output in ewes. *Arkansas Farm Research* **31**: 9.

Strahan, S.R.; Hemken, R.W.; Jackson Jr, J.A.; Buckner, R.C.; Bush, L.P.; Siegel, M.R. 1987. Performance of lactating dairy cows fed tall fescue forage. *Journal of Dairy Science* **70**: 1228-1234.

Stuedemann, J.A.; Breedlove, D.L.; Pond, K.R.; Belesky, D.P.; Tate, L.P. Jr.; Thompson, F.N.; Wilkinson, S.R. 1989. Effect of endophyte (Acremonium coenophialum) infection of tall fescue and paddock exchange on intake and performance of grazing steers. Proceedings of the XVI International Grassland Congress, 4-11 October 1989, Nice, France. 1989, 1243-1244;

Suescun, M.O; Gonzalez, S.I.; Chiauzzi, V.A.; Calandra, R.S. 1985. Effects of induced hypoprolactinemia on testicular fuction during gonadal maturation in the rat. *Journal of. Andrology* **6**:77-82.

Sutherland, B.L.; Hoglund, J.H. 1989. Effect of ryegrass containing the endophyte (*Acremonium lolii*), on the performance of associated white clover and subsequent crops. *Proceedings of the New Zealand Grassland Association* **50**: 265-269.

Szafranska, B.; Grazul-Bilska, A.; Przala, J. 1992. Effect of LH and prolactin on steroid secretion by perifused luteal tissue from pregnant gilts with induced hypoprolactinemia or after passive immunoneutralization if LH. *Polskie Archiwum Weterynaryjne* **32**: 1-2, 7-15.

Taylor, M.C.; Loch, W.E.; Ellersieck, M. 1985. Toxicity in pregnant pony mares grazing Kentucky-31 fescue pastures. *Nutrition Reports International* **31**:787.

Thom, E.R.; Clark, D.A.; Prestige, R.A.; Clarkson, F.H.; Waugh, C.D. 1994. Ryegrass endophyte, cow health and milksolids production for the 1993/94 season. *Proceedings of the New Zealand Grassland Association* **56**: 259-264.

Thompson, G.E.; Bassett, J.M.; Samson, D.E.; Slee, J. 1982. The effects of cold exposure of pregnant sheep on foetal plasma nutrients, hormones and birth weight. *British Journal of Nutrition* **48**: 59-64

Thompson, C.H.; Thompson, D.L.; Kincaid, L.A.; Nadal, R. 1996. Prolactin involvement with the increase in seminal volume after sexual stimulation in stallions. *Journal of Animal Science* **74**:2468-2472.

Thompson, F.N.; Stuedemann, J.A. 1993. Pathophysiology of fescue toxicosis. P. 263-281. *In* R. Joost and S. Quisenberry (eds.). *Acremonium*/Grass Interactions. Elsevier, The Netherlands.

Thompson, F.N.; Stuedemann, J.A.; Sartin, J.L.; Belesky, D.P.; Devine, O.J. 1987. Selected hormonal changes with summer fescue toxicosis. *Journal of Animal Science* **65**: 727-733.

Thwaits, C.J. 1971. Short term heat tress and embryo mortality in the ewe. *Australian Journal of Experimental Agriculture and Animal Husbandry* **11**: 265-267.

Tucker, C.A.; Morrow, R.E.; Gerrish, J.R.; Nelson, C.J.; Garner, G.B.; Jacobs, V.E.; Hires, W.G.; Shinkle, J.J.; Forwood, J.R. 1989. Forage systems for beef cattle: Effect of winter supplementation and forage system on reproductive performance of cows. *Journal of Production Agriculture* **2**:217.

Tverdeic, A.; Pericic, D. 1991. Dihydrogenated ergot compounds bind with high affinity to GABAa receptor-associated CL-ionophore. *European Journal of Pharmacology* **202**: 109-111.

Valentine, S.C.; Bartsch, B.D.; Carroll, P.D. 1993. Production and composition of milk by dairy cattle grazing high and low endophyte cultivars of perennial ryegrass. P 138-141. In Proceedings of the 2nd International Symposium on *Acremonium*/Grass Interactions. D.E. Hume; G.C.M. Latch; Easton, H.S. (eds.)

Van Heeswijk, R.; McDonald, G. 1992. *Acremonium* endophytes in perennial ryegrass and other pasture grasses in Australia and New Zealand. *Australian Journal of Agricultural Research* **43**: 1608-1709.

Van Landeghem, A.A.J.; Van de Wiel, D.F.M. 1978. Radioimmunoassay for porcine prolactin: plasma levels during lactation, suckling and weaning and after TRH administration. *Acta Endocrinologica* **88**: 4, 653-667.

Varney, D.R.; Ndefru, M.; Jones, S.L.; Newsome, R.; Siegel, M.R.; Zavos, P.M. 1987. The effect of feeding endophyte infected tall fescue seed on reproductive performance in female rats. *Comparative Biochemistry and Physiology* **87C**, No.1, pp. 171-175.

Varney, D.R.; Varney, L.A.; Hemken, R.W.; Zavos, P.M.; Siegel, M.R. 1991a. Onset of puberty in CD-1 mouse pups exposed prenatally through weaning to endophyte-infected tall fescue seed. *Theriogenology* **35**, NO.5: 883-892.

Varney, D.R.; Varney, L.A.; Hemken, R.W.; Zavos, P.M.; Wiglesworth, M.D.; Siegel, M.R. 1991b. Tall fescue endophyte: Effect on congenital development and pup growth in mice. *Journal of Dairy Science* **74**: 460-466.

Vernon, R.G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. *Progress in Lipid Research* **19**: 23-106.

Vernon, R.G. 1982. Effects of growth hormone on fatty acid synthesis in sheep adipose tissue. *International Journal of Biochemistry* **14**: 255-258.

Vernon, R.G.; Clegg, R.A.; Flint, D.J. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation: adaption and regulation. *Biochemical Journal* **200**: 307-314.

Vivrette, S. 1994. The endocrinology of parturition in the mare. *Veterinary Clinics of North American: Equine Practice* **10:** 1.

Washburn, S.P.; Green, J.T. 1991. Performance of replacement beef heifers on endophyte-infected fescue pastures. In: Proceedings of the 40th Annual Conference. North Carolina Cattlemen's Assoc. February 25-26, 1991. North Carolina State Univ., Raleigh.

Washburn, S.P.; Green, J.T.; Johnson, B.H. 1989. Effects of endophyte presence in tall fescue on growth, puberty, and conception in Angus heifers. In: Proceedings of the Tall Fescue Toxicosis Workshop. November 13-14. p 80. Southern Region Information Exchange Group 37. Atlanta, GA.

Watson, R.H.; Keogh, R.G.; McDonald, M.F. 1999. Ewe reproductive performance and growth rate of suckling-lambs on endophyte-infected perennial ryegrass pasture. *Ryegrass endophyte: an essential New Zealand symbiosis. Grassland Research and Practice Series No. 7*: 19-28.

Wedderburn, M.E.; Pengelly, W.J.; Tucker, M.A.; di Menna, M.E. 1989. Description of ryegrass removed from New Zealand North Island hill country. *New Zealand Journal of Agricultural Research* **32**: 521-529.

Weiland, N.G.; Wise, P.M. 1987. Estrogen alters the diurnal rhythm of alpha1-adrenegic receptor densities in selected brain regions. *Endocrinology* **121**: 1751-1758.

Whitaker-Azmitia, P.M.; Peroutka, S.J. (eds.). 1990.The neuropharmacology of serotonin. *Annals of the New York Academy of Sciences.*

Widdup, K.H.; Ryan, D.L. 1992. Forage potential of wild populations of perennial ryegrass collected from southern New Zealand farms. *Proceedings of the New Zealand Grassland Association* **54**: 161-165.

Williams, M.J.; Backman, P.A.; Crawford, M.A.; Schmidt, S.P.; King, C.C. 1984. Chemical control of the tall fescue endophyte and its relationship to cattle performance. *New Zealand Journal of Experimental Agriculture* **12**: 165.

Zampa, G.A.; Benfenati, F.; Ghisoli, E.; Corbucci, G.; Vecchi, P.; Zini, I.; Battistini, N.; Agnati, L.F. 1981. Neuroendocrine control of basal insulin secretion in man: a study with bromocriptine, clonidine and naloxone. *Journal of Endocrinological Investigation* **4**: 423-429.

Zavos, P.M.; Salim, B.; Jackson, J.A.; Varney, D.R.; Siegel, M.R.; Hemken, R.W. 1986. Effect of feeding tall fescue seed infected by endophytic fungus (*Acremonium coenophialum*) on reproductive performance in male rats. *Theriogenology* **25**:281-290.

Zavos, P.M.; Varney, D.R.; Jackson, J.A.; Siegel, M.R.; Bush, L.P.; Hemken, R.W. 1987a. Effect of feeding endophyte (Acremonium coenophialum) infected tall fescue seed on reproductive performance of CD-1 mice through continuous breeding. *Theriogenology* **27**: 549-559.

Zavos, P.M.; Varney, D.R.; Siegel, M.R.; Hemken, R.W.; Jackson, J.A.; Bush, L.P. 1987b. Effects of feeding endophyte-infected tall fescue seed on the reproductive performance in male and female CD-1 mice via combination crosses. *Theriogenology* **27**: 541-548.