

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

# **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 milk solid 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 the 1998 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 ( $30^{\circ}\text{C}$ ) ambient temperature. Serum progesterone levels were ( $P < 0.001$ ) lower in Ev+ than in Ev- ewes at both high and low ( $18^{\circ}\text{C}$ ) 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 1 and 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

Abstract .....	ii
Acknowledgements.....	vi
List of tables .....	xiv
List of figures .....	xix
List of plates .....	xxii
List of abbreviations and definitions.....	xxiii
<b>CHAPTER I: General introduction .....</b>	<b>1</b>
<b>CHAPTER II: Review of literature .....</b>	<b>4</b>
1. Introduction.....	4
2. Effects of endophyte on equine fertility .....	4
3. Effects of endophyte on bovine fertility .....	6
4. Effects of endophyte on laboratory animal fertility.....	8
4.1. Rats.....	8
4.2. Mice.....	8
4.3. Rabbits.....	9
5. Effects of endophyte on deer fertility.....	9
6. Effects of endophyte on ovine fertility.....	10
7. Mechanisms by which endophyte reduces fertility.....	10
7.1. Pharmacological and physiological effects of endophyte toxins.....	10
7.1.1. Ergopeptine alkaloids.....	11
7.1.2. Lysergic acid amides.....	12
7.1.3. Lolines.....	12
7.1.4. Lolitrems.....	13
7.1.5. Alkaloid synergism.....	13
7.2. Dopaminergic effects of endophyte toxins.....	13
7.3. Serotonergic (5HT) effects of endophyte toxins.....	14
7.4. $\alpha$ -adrenergic effects of endophyte toxins.....	15
7.5. Prolactin suppression by endophyte toxins.....	15
7.5.1. Prolactin and milk production in animals grazing E+ feed.....	17
7.6. Effects of endophyte toxins on luteinizing hormone (LH) secretion.....	18
7.7. Effects of endophyte toxins on progesterone secretion.....	19
7.8. Effects of endophyte toxins on cortisol secretion.....	20
7.9. Effects of endophyte toxins on estradiol $17\beta$ secretion.....	20
7.10. Effects of endophyte toxins on gamma-aminobutyric acid (GABA) secretion.....	20
8. Indirect effects of endophyte toxicosis on reproduction.....	21
8.1. Heat stress.....	21
8.2. Nutrition.....	23
8.2.1. Feed intake and grazing behaviour.....	23
8.2.2. Forage digestibility.....	24
8.2.3. Effects of endophyte on the gastrointestinal (GI) system.....	24
9. Production of the major endophyte toxins in E+ perennial ryegrass pasture.....	25
9.1. Lolitrem B.....	25

9.2. Ergovaline.....	25
10. Purpose and scope of the study.....	26

**CHAPTER III: Reproductive performance of ewes grazing either endophyte-infected or endophyte-free perennial ryegrass.....27**

1. Introduction.....	27
2. Materials and methods.....	28
2.1. Animals and treatments.....	28
2.1.1. Animals.....	28
2.1.2. Treatment pastures.....	28
2.1.3. Fertiliser applications.....	29
2.1.4. Flock management.....	29
2.2. Sampling.....	31
2.2.1. Animal measurements.....	31
2.2.2. Pasture measurements.....	32
2.3. Endophyte infection levels.....	34
2.4. Serum prolactin analysis.....	34
2.5. Herbage ergovaline and lolitrem B analyses.....	34
2.6. Pasture nutritional analysis.....	35
2.7. Statistical analyses.....	35
3. Results.....	36
3.1. Animal measurements.....	36
3.1.1. Reproductive performance and ewe mortality.....	36
3.1.2. Reproductive performance for the whole trial period.....	38
3.1.3. Serum prolactin.....	39
3.1.4. Ewe liveweight.....	40
3.1.5. Relationships between ewe liveweight at mating, liveweight change during mating and reproductive performance.....	44
3.1.6. Daily milksolid production and cumulative milksolid yield .....	47
3.1.7. Lamb birthweight.....	50
3.1.8. Lamb growth rate.....	50
3.1.9. Relationship between milk production and lamb growth rate.....	52
3.1.10. Ewe greasy fleeceweight.....	53
3.1.11. Faecal soiling (dags).....	54
3.1.12. Ryegrass staggers and lethargy.....	54
3.1.13. Body temperature.....	54
3.1.14. Urinary zearalenone.....	55
3.2. Pasture measurements.....	55
3.2.1. Pasture growth rate.....	55
3.2.2. Pasture ergovaline and lolitrem B levels.....	56
3.2.3. Endophyte infection levels.....	58
3.2.4. Pasture nutritional analysis.....	58
3.2.5. Pasture postgrazing residues.....	59
4. Discussion and conclusions.....	59
4.1. Reproductive performance.....	59
4.2. Ewe liveweight.....	62
4.3. Milk production.....	65
4.4. Lamb growth rate.....	65
4.5. Wool production and faecal soiling.....	67

4.6. Body temperature, ryegrass staggers and lethargy.....	68
4.7. Zearalenone.....	69
4.8. Pastures.....	70
4.9. Conclusions.....	71

#### **CHAPTER IV: Effects of endophyte-infected perennial ryegrass on ewe fertility in a Northland environment.....72**

1. Introduction.....	72
2. Materials and methods.....	73
2.1. 1998 Trial.....	73
2.1.1. Animals.....	74
2.1.2. Treatment pastures.....	74
2.1.3. Flock management.....	74
2.2. 1999 Trial.....	78
2.2.1. Animals.....	78
2.2.2. Flock management.....	80
2.3. Sampling.....	81
2.3.1. Blood.....	81
2.3.2. Herbage.....	81
2.4. Prolactin analysis.....	81
2.5. Herbage ergovaline and lolitrem B analyses.....	81
2.6. Statistical analyses.....	82
3. Results.....	82
3.1. Reproductive performance.....	82
3.2. Ewe liveweight.....	83
3.3. Relationship between ewe liveweight, pre-mating liveweight change and reproductive performance.....	84
3.4. Serum prolactin.....	88
3.5. Serum GGT.....	88
3.6. Relationship between serum GGT and reproductive performance.....	88
3.7. Herbage ergovaline and lolitrem B concentration.....	91
3.8. Pasture nutritional analysis.....	92
4. Discussion and conclusions.....	93
4.1. Reproductive performance and trial limitations.....	93
4.2. Ewe liveweight, liveweight change and their relationship with reproductive performance.....	95
4.3. Serum prolactin.....	96
4.4. Serum GGT.....	98
4.5. Pastures and insect pests.....	98
4.6. Conclusions.....	99

#### **CHAPTER V: Ewe liveweight, lamb birthweight and liveweight gain during lactation on a Northland endophyte-infected ryegrass pasture.....101**

1. Introduction.....	101
2. Materials and methods.....	102
2.1. Animals and treatments.....	102
2.1.1. Animals.....	102
2.1.2. Treatment pastures.....	102

2.2. Sampling.....	102
2.2.1. Animal measurements.....	102
2.2.2. Pasture measurements.....	103
2.2.3. Prolactin analysis.....	103
2.2.4. Ergovaline and lolitrem B analyses.....	103
2.2.5. Pasture nutritional analysis.....	103
2.2.6. Statistical analyses.....	104
3. Results.....	104
3.1. Ewe liveweight.....	104
3.2. Lambing date and birthweight.....	105
3.3. Lamb growth rate.....	106
3.4. Lamb weaning weights.....	107
3.5. Lamb survival.....	108
3.6. Serum prolactin.....	109
3.7. Pasture dry matter cover.....	109
3.8. Herbage ergovaline and lolitrem B concentration.....	110
3.9. Pasture nutritional analysis.....	110
4. Discussion and conclusions.....	111
4.1. Ewe liveweight.....	111
4.2. Lamb birthweight, growth rate and survival.....	113
4.3. Conclusions.....	115

**CHAPTER VI: Effects of long-term grazing of endophyte-infected perennial ryegrass on serum luteinizing hormone, progesterone and prolactin levels in cyclic ewes..... 116**

1. Introduction.....	116
2. Materials and methods.....	117
2.1. Animals and treatments.....	117
2.2. Sampling.....	117
2.2.1. Blood.....	117
2.2.2. Rectal temperature.....	118
2.2.3. Herbage.....	118
2.3. Hormone analyses.....	118
2.3.1. Progesterone.....	118
2.3.2. Prolactin.....	119
2.3.3. Luteinizing hormone (LH).....	120
2.4. Statistical analyses.....	122
3. Results.....	122
3.1. Serum progesterone.....	122
3.2. Serum luteinizing hormone (LH).....	123
3.3. Serum prolactin.....	124
3.4. Ambient temperature and ewe body temperature.....	124
3.5. Pasture ergovaline and lolitrem B levels.....	125
4. Discussion and conclusions.....	125
4.1. Serum progesterone.....	125
4.2. Serum luteinizing hormone.....	126
4.3. Serum prolactin.....	127
4.4. Conclusions.....	129

<b>CHAPTER VII: Effects of ergovaline and ambient temperature on selected hormones associated with mammary development in the ewe, and on lamb performance.....</b>	<b>130</b>
1. Introduction.....	130
2. Materials and methods.....	132
2.1. Trial 1.....	132
2.1.1. Animals.....	132
2.1.2. Treatments.....	132
2.1.3. Sampling and measurements.....	134
2.2. Trial 2.....	134
2.2.1. Animals.....	134
2.2.2. Treatments.....	135
2.2.3. Sampling and measurements.....	135
2.3. Faecal moisture.....	136
2.4. Serum prolactin analysis.....	136
2.5. Serum progesterone analysis.....	136
2.6. Serum cortisol analysis.....	137
2.7. Serum insulin analysis.....	137
2.8. Statistical analyses.....	137
3. Results.....	138
3.1. Trial 1.....	138
3.1.1. Serum prolactin.....	138
3.1.2. Milk constituents.....	139
3.1.3. Birthweight and lamb growth rate.....	139
3.1.4. Ewe rectal temperature.....	140
3.1.5. Relationship between rectal temperature and daily ergovaline intake.....	141
3.1.6. Feed intake.....	142
3.1.7. Water intake.....	142
3.2. Trial 2.....	143
3.2.1. Serum prolactin.....	143
3.2.2. Serum progesterone.....	145
3.2.3. Serum cortisol.....	146
3.2.4. Serum insulin.....	147
3.2.5. Rectal temperature.....	148
3.2.6. Milk production and mammary development.....	149
3.2.7. Lamb growth rate.....	149
3.2.8. Maternal behaviour.....	149
3.2.9. Faecal moisture.....	150
4. Discussion and conclusions.....	153
4.1. Serum prolactin.....	153
4.2. Serum progesterone.....	154
4.3. Serum cortisol.....	156
4.4. Serum insulin.....	156
4.5. Mammogenesis and lactogenesis.....	158
4.6. Thermoregulation.....	161
4.7. Maternal behaviour.....	162
4.8. Faecal moisture.....	163
4.9. Conclusions.....	164

**CHAPTER VIII: Feed intake and grazing behaviour of ewes and lambs grazing either endophyte-infected or endophyte-free perennial ryegrass.....165**

1.	Introduction.....	165
2.	Materials and methods.....	166
	2.1. Animals.....	166
	2.2. Grazing management.....	166
	2.3. Use of controlled release chromium oxide capsules.....	169
	2.4. Faecal sampling.....	169
	2.5. Herbage sampling.....	170
	2.6. Herbage ergovaline and lolitrem B analyses.....	172
	2.7. Chromium analysis.....	172
	2.8. Statistical analyses.....	172
3.	Results.....	173
	3.1. Faecal chromium oxide levels.....	173
	3.1.1. Ewes.....	173
	3.1.2. Lambs.....	174
	3.2. Ewe liveweight and liveweight change.....	175
	3.3. Lamb liveweight and liveweight change.....	175
	3.4. Relationship between ewe liveweight, liveweight change and faecal chromium levels.....	176
	3.5. Relationship between lamb liveweight, growth rate, and faecal chromium levels.....	177
	3.6. Pasture growth rate.....	178
	3.7. Pasture nutritional analysis.....	179
	3.8. Ergovaline and lolitrem B levels in the pasture and distribution within the ryegrass plant.....	179
	3.9. Pre- and post-grazing tiller dimensions.....	180
	3.10. Effects of grazing on pasture botanical composition.....	183
4.	Discussion and conclusions.....	185
	4.1. Faecal chromium and feed intake.....	185
	4.2. Grazing behaviour.....	187
	4.3. Conclusions.....	189

**CHAPTER IX: General discussion and conclusions.....190**

1.	Reproductive performance of the ewe.....	190
2.	Effects of endophyte on hormones associated with reproduction in the ewe.....	192
3.	Endophytic ryegrass and ram fertility.....	194
4.	Ewe liveweight, lamb growth rate, and feed intake.....	195
5.	Grazing behaviour and pasture composition.....	196
6.	Milk production, mammary development and maternal behaviour.....	198
7.	Conclusions.....	199

**Appendices.....201**

**References.....207**

## LIST OF TABLES

<u>TABLE</u>	<u>PAGE</u>
<b>Table 2.1.</b> Effects of consuming tall fescue infected with endophyte fungi on pregnant mares and their foals (Putnam <i>et al.</i> , 1991).....	6
<b>Table 2.2.</b> 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)).....	19
<b>Table 3.1.</b> Fertiliser applications made during the pre-trial period and during 1997 and 1998.....	29
<b>Table 3.2.</b> Ovulation rate, % lambs carried at scanning, % of ewes that returned to oestrus and that were dry at scanning in the E+ and E- groups.....	37
<b>Table 3.3.</b> Mean ( $\pm$ SEM) number of days from the start of mating until ewes in the E+ and E- groups were mated in each year (1997, 1998).....	37
<b>Table 3.4.</b> Mean ( $\pm$ 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 1999.....	38
<b>Table 3.5.</b> 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.....	39
<b>Table 3.6.</b> Mean ( $\pm$ SEM) liveweight (kg) of twin and single-rearing ewes grazing either E+ or E- perennial ryegrass pasture in each year (1997, 1998) .....	43
<b>Table 3.7.</b> Mean ( $\pm$ SEM) ewe liveweight (WT) (kg) and liveweight change (LW $\Delta$ ) (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).....	46
<b>Table 3.8.</b> Mean ( $\pm$ SEM) daily milk solid production (g/d) for E+ and E- twin and single-rearing ewes during the first 12 weeks of lactation in 1997.....	48

<u>TABLE</u>	<u>PAGE</u>
<b>Table 3.9.</b> Mean ( $\pm$ 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.....	48
<b>Table 3.10.</b> Mean ( $\pm$ SEM) birth weights (kg) of twin and single lambs born to ewes grazing either E+ or E- ryegrass.....	50
<b>Table 3.11.</b> Slope ( $\pm$ SEM) and $r^2$ for the regression analysis of cumulative milksolid and lamb liveweight for each group in 1997.....	53
<b>Table 3.12.</b> Mean ( $\pm$ SEM) greasy fleece-weight (kg) of ewes grazing either E+ or E- ryegrass (1997, 1998).....	53
<b>Table 3.13.</b> Mean ( $\pm$ SEM) faecal soiling scores of E+ and E- ewes during summer and autumn of (1997, 1998).....	54
<b>Table 3.14.</b> Mean ( $\pm$ SEM) rectal temperature ( $^{\circ}$ C) of E+ and E- ewes at different ambient temperature ranges.....	55
<b>Table 3.15.</b> Mean ( $\pm$ SEM) nutritional parameters for the E+ and E- ryegrass pasture.....	58
<b>Table 3.16.</b> Mean ( $\pm$ SEM) pre-grazing pasture allowance and post grazing residues of the E+ and E- groups in spring, summer and autumn in each year (1997, 1998).....	59
<b>Table 4.1.</b> Mean ( $\pm$ 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.....	82
<b>Table 4.2.</b> Mean ( $\pm$ 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.....	83
<b>Table 4.3.</b> Mean ( $\pm$ SEM) liveweight (LW), pre-mating liveweight change (LW $\Delta$ ) and numbers of ewes of different ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) within the E+ and E- groups in the 1998 trial.....	86

**TABLE****PAGE**

<b>Table 4.4.</b>	Numbers and liveweight (WT) of ewes in each group of different mean ( $\pm$ SEM) ovulation rate (OR), scanning rank (SCN) and cycle of conception (CYC) within the short- and long-exposure, E+ and E- groups in the 1999 trial.....	<b>87</b>
<b>Table 4.5.</b>	Mean serum GGT 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.....	<b>90</b>
<b>Table 4.6.</b>	Mean serum GGT levels 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.....	<b>91</b>
<b>Table 4.7.</b>	Mean ( $\pm$ SEM) nutritional parameters in the E+ and E-pasture during mating in 1999.....	<b>93</b>
<b>Table 5.1.</b>	Mean ( $\pm$ 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.....	<b>105</b>
<b>Table 5.2.</b>	Mean ( $\pm$ SEM) birthweight (kg) and the number (N) of ram and ewe lambs born to ewes grazing either endophyte-infected (E+) or endophyte-free (E-) pasture.....	<b>105</b>
<b>Table 5.3.</b>	Mean ( $\pm$ 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.....	<b>107</b>
<b>Table 5.4.</b>	Mean ( $\pm$ 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.....	<b>108</b>
<b>Table 5.5.</b>	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- groups.....	<b>109</b>

<b><u>TABLE</u></b>	<b><u>PAGE</u></b>
<b>Table 5.6.</b> Mean ( $\pm$ SEM) nutritional parameters in Northland E+ and E- ryegrass pasture.....	111
<b>Table 6.1.</b> Progesterone standards.....	119
<b>Table 6.2.</b> Luteinizing hormone standards.....	121
<b>Table 7.1.</b> Mean ( $\pm$ SEM) milk fat, protein, lactose and total milk solids at days 5, 10, 15 and 20 of lactation in ewes fed a diet with (Ev+) or without (Ev-) ergovaline.....	139
<b>Table 8.1.</b> Mean ( $\pm$ SEM) faecal chromium oxide levels ( $\mu$ g/g) for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.....	173
<b>Table 8.2.</b> Mean ( $\pm$ SEM) faecal chromium oxide levels ( $\mu$ g/g) for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.....	174
<b>Table 8.3.</b> Mean ( $\pm$ SEM) pre- (PRE-LW) and post-trial (POST-LW) liveweight (kg) and liveweight change ( $\Delta$ LW) (g/d) of twin and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.....	175
<b>Table 8.4.</b> Mean ( $\pm$ SEM) pre- (PRE-LW) and post-trial (POST-LW) liveweight (kg) and liveweight change ( $\Delta$ LW) (g/d) of twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.....	176
<b>Table 8.5.</b> Mean ( $\pm$ SEM) faecal chromium oxide levels ( $\mu$ g/g), adjusted for pre-trial ewe liveweight, for twin- and single-rearing ewes grazing either endophyte-infected or endophyte-free ryegrass pasture.....	177

<b><u>TABLE</u></b>	<b><u>PAGE</u></b>
<b>Table 8.6.</b> Mean ( $\pm$ SEM) faecal chromium oxide levels ( $\mu\text{g/g}$ ), adjusted for lamb pre-trial liveweight, for twin and single lambs grazing either endophyte-infected or endophyte-free ryegrass pasture.....	178
<b>Table 8.7.</b> 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.....	180
<b>Table 8.8.</b> Mean ( $\pm$ SEM) pre- and post-grazing weights ( $\mu\text{g}$ 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.....	182
<b>Table 8.9.</b> 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.....	184
<b>Appendix Table 1.1.</b> Number and cause of ewe deaths in the E+ and E- groups during 1997 and 1998 in the Manawatu trial.....	201

## LIST OF FIGURES

<u>FIGURE</u>	<u>PAGE</u>
<b>Figure 3.1.</b> Mean ( $\pm$ SEM) serum prolactin levels of single and twin-bearing ewes grazed on either E+ or E- ryegrass.....	40
<b>Figure 3.2.</b> Mean ( $\pm$ SEM) cumulative milksolid yield for twin- and single-rearing ewes in the E+ and E- groups during the first 12 weeks of lactation in 1997.....	49
<b>Figure 3.3.</b> Mean ( $\pm$ SEM) cumulative milksolid yield for twin- and single-rearing ewes in the E+ and E- groups during the first 5 weeks of lactation in 1998.....	49
<b>Figure 3.4.</b> 1997 mean ( $\pm$ SEM) daily growth rates of twin and single lambs born to ewes grazing either E+ or E- ryegrass.....	51
<b>Figure 3.5.</b> 1998 mean ( $\pm$ SEM) daily growth rates of twin and single lambs born to ewes grazing either E+ or E- ryegrass.....	52
<b>Figure 3.6.</b> Mean ( $\pm$ SEM) daily pasture growth rate of E+ and E- ryegrass pastures between March 1997 and December 1998.....	56
<b>Figure 3.7.</b> Mean ( $\pm$ SEM) ergovaline concentrations in the leaf blade and leaf sheath components of the E+ pasture during 1997 and 1998.....	57
<b>Figure 3.8.</b> Mean ( $\pm$ SEM) lolitrem B concentrations in the leaf blade and leaf sheath components of the E+ pasture during 1997 and 1998.....	57
<b>Figure 6.1.</b> Mean ( $\pm$ SEM) serum progesterone levels in cyclic ewes grazing either E+ or E-perennial ryegrass pasture.....	123
<b>Figure 6.2.</b> Mean ( $\pm$ SEM) serum LH levels in cyclic ewes grazing either E+ or E-perennial ryegrass pasture.....	123

<b><u>FIGURE</u></b>	<b><u>PAGE</u></b>
<b>Figure 6.3.</b> Mean ( $\pm$ SEM) serum prolactin levels in cyclic ewes grazing either E+ or E-perennial ryegrass pasture.....	124
<b>Figure 7.1.</b> Mean ( $\pm$ SEM) serum prolactin concentration in ewes feed diets either with (Ev+) or without (Ev-) ergovaline (Trial 1).....	138
<b>Figure 7.2.</b> Mean ( $\pm$ SEM) daily growth rate of lambs born to ewes with (Ev+) or without (Ev-) ergovaline in their diet (Trial 1).....	140
<b>Figure 7.3.</b> Mean ( $\pm$ SEM) rectal temperatures in ewes offered diets with (Ev+) or without (Ev-) ergovaline (Trial 1).....	141
<b>Figure 7.4.</b> Relationship between daily ergovaline intake and rectal temperature in ewes (Trial 1).....	141
<b>Figure 7.5.</b> Mean ( $\pm$ SEM) daily total dry matter intake of ewes fed diets with (Ev+) or without (Ev-) ergovaline during late pregnancy (Trial 1).....	142
<b>Figure 7.6.</b> Mean ( $\pm$ SEM) daily water consumption of ewes fed diets with (Ev+) or without (Ev-) ergovaline during late pregnancy (Trial 1).....	143
<b>Figure 7.7.</b> Mean ( $\pm$ SEM) serum prolactin levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline and maintained at high and low ambient temperature (Trial 2).....	144
<b>Figure 7.8.</b> Mean ( $\pm$ SEM) serum progesterone levels in ewes fed a diet with (Ev+) or without (Ev-) ergovaline, and maintained at high or low ambient temperature (Trial 2).....	145
<b>Figure 7.9.</b> Mean ( $\pm$ SEM) serum cortisol levels in ewes fed diets with (Ev+) or without (Ev-) ergovaline, and maintained at high or low ambient temperature (Trial 2).....	146
<b>Figure 7.10.</b> Serum insulin levels in the Ev+ and Ev- ewes in the high and low temperature rooms (Trial 2).....	147

**FIGURE****PAGE**

- Figure 7.11.** Mean ( $\pm$ SEM) rectal temperature in ewes fed a diet with (Ev+) or without (Ev-) ergovaline at high and low ambient temperature (Trial 2).....148
- Figure 7.12.** Faecal moisture in a ewe that was fed Ev+ diet than Ev- diet and a ewe that was fed the Ev- diet then the Ev+ diet.....150

## LIST OF PLATES

<b><u>PLATE</u></b>	<b><u>PAGE</u></b>
<b>Plate 3.1.</b> Faecal soiling of the wool around the crutch commonly referred to as dags.....	33
<b>Plate 4.1.</b> Black cricket ( <i>T. commodus</i> ) in the E- treatment pasture.....	75
<b>Plate 4.2.</b> E- pasture during mating 1998.....	76
<b>Plate 4.3.</b> E+ pasture during mating 1998.....	76
<b>Plate 4.4.</b> Laparoscopic examination of ewe ovaries to determine ovulation rate.....	77
<b>Plate 4.5.</b> E- pasture during mating 1999.....	79
<b>Plate 4.6.</b> E+ pasture during mating 1999.....	79
<b>Plate 4.7.</b> Grazed tall fescue plants in the E- pasture.....	97
<b>Plate 7.1.</b> Housing arrangement for ewes.....	133
<b>Plate 7.2.</b> Mammary gland of EV+ low temperature ewe.....	151
<b>Plate 7.3.</b> Mammary gland of EV- low temperature ewe.....	151
<b>Plate 7.4.</b> Mammary gland of EV+ high temperature ewe .....	152
<b>Plate 7.5.</b> Mammary gland of EV- high temperature ewe. ....	152
<b>Plate 8.1.</b> Ewes and lambs grazing treatment pastures.....	167
<b>Plate 8.2.</b> Pre-grazing E+ pasture.....	168
<b>Plate 8.3.</b> Pre-grazing E- pasture.....	168
<b>Plate 8.4.</b> Ryegrass tiller dissection.....	171

## 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- $\alpha$ -ergocriptine (bromocriptine)
CHO	soluble carbohydrate
CIDR	controlled internal drug release (device)
CNS	central nervous system
CV	co-efficient of variation
<i>cv</i>	cultivar
$^{\circ}\text{C}$	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
LW $\Delta$	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.