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## Ergot alkaloids in New Zealand pastures and their impact

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### ABSTRACT

In identifying endophytes for use in pastures in New Zealand there have been two strategies used, either exclusion of ergopeptine and lolitrem alkaloids or, retaining some ergopeptine expression to enhance the stability of natural defences against invertebrate pests. Both have their strengths and weaknesses. It is a matter of balancing these to ensure the end-user has access to a product that satisfies their needs – a persistent pasture with low to nil animal toxicity in terms of animal production and welfare. The range and intensity of ergot alkaloids in grazed pasture on both pasture and ruminants is reviewed, with emphasis on New Zealand. Ergot alkaloids associated with pasture are produced by associations between certain *Epichloë* endophyte strains and temperate grasses. Ergot alkaloids have been shown to improve persistence of pasture through providing resistance/deterrence to insect pests as well as deterring grazing animals. However, ergovaline is toxic to grazing animals. Some commercially available ryegrass-endophyte associations can produce ergovaline concentrations close to those found in associations between ryegrass and the standard endophyte. It is feasible to eliminate ergot alkaloids from pasture grasses in New Zealand as endophyte strains are commercially available that do not express ergovaline and yet still provide excellent pest resistance.

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## Introduction

Pasture species from Europe, mainly England, were purposefully brought to New Zealand in the nineteenth century as the early European settlers experimented with various forages in different parts of the country primarily for wool, meat and dairy production (Cockayne 1912; Daly 1973; Stewart 2006). Grass species (family Poaceae) that were trialled included Cock's-foot (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), fine fescues (*Festuca ovina*, *F. rubra* and *F. rubra* var. *commutata*) (Star and Brooking 2006) and tall fescue (*F. arundinacea*) which arrived later via Australia (Easton et al. 1994).

Fungal endophyte species of the *Epichloë* genus (family Clavicipitaceae), previously known as *Acremonium* and *Neotyphodium*, reside in the seed of several of grass species and were unknowingly introduced to New Zealand when grass seed was imported (Johnson et al. 2013).

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*Epichloë* spp. are largely seed transmitted, obligate mutualists that may confer advantageous traits to their host and allow the plant to be more resilient to some abiotic stresses, such as drought (Hume et al. 1993; Malinowski and Belesky 2000; He et al. 2013).

*Epichloë* endophytes are known to produce four major classes of bioactive alkaloids. These are the ergot alkaloids which include ergovaline, the indole diterpenes which include lolitrem B (Gallagher et al. 1984; Miles et al. 1994) and epoxy janthitrems (Finch et al. 2012, 2013), pyrrolizidines which include the lolines, and the pyrrolopyrazine metabolites which include peramine (Porter 1995; Siegel and Bush 1996; Lane et al. 2000; Schardl et al. 2013; Panaccione et al. 2014; Berry et al. 2019). Different ergot alkaloids are produced by several fungi in nine genera in the Clavicipitaceae, Aspergillaceae, Arthrodermataceae (Trichocomaceae) families (Gerhards et al. 2014), although all have the same early steps before their pathways diverge to produce different end products (Robinson and Panaccione 2015). Specifically, in the Clavicipitaceae to which the *Epichloë* belong, Robinson and Panaccione (2015) noted that diversity is generated by the presence or absence of lysergyl peptide synthetases, which interact to make lysergic acid amides and ergot alkaloids. The diversity of ergot alkaloids has resulted from gene gains, gene losses, and gene sequence changes during the evolutionary development of *Epichloë* (Young et al. 2015).

In New Zealand, the naturally-occurring standard endophyte in ryegrass, *Epichloë festucae* var. *lolii*, expresses a wide range of metabolites. Some of these compounds, such as peramine, (Lane et al. 2000; Schardl et al. 2013) are beneficial to the pastoral system as they protect the host plant from herbivory (Hume et al. 2009; Johnson et al. 2013). However, other endophyte-expressed metabolites, such as lolitrem B and ergot alkaloids are detrimental (Semán et al. 1990) as they are associated with animal diseases including ryegrass staggers, fescue toxicosis (Hemken et al. 1984) and heat stress (Prestidge 1993; Strickland et al. 2011; di Menna et al. 2012) as well as losses in animal production (Schmidt and Osborn 1993; Browning 2000; Waller 2009; Strickland et al. 2011).

Under New Zealand environmental conditions, the presence of an endophyte is essential due to insect pest pressures, but the detrimental effects of endophytes can be minimised by identifying and selecting endophytes with a favourable chemical profile and inoculating these endophytes into modern grass cultivars (Johnson et al. 2013). It is widely accepted that the elimination of lolitrem B, the causative agent of ryegrass staggers, is desirable due to the significant impacts of ryegrass staggers on animal health and welfare (Fletcher and Harvey 1981). Although it is also possible to eliminate ergot alkaloids, the causative agent of several animal health and welfare issues including heat stress (Eady et al. 2017) from New Zealand pastoral systems, there is not a clear consensus among New Zealand seed companies marketing endophytes as to whether this is the best strategy. Of all the ergot alkaloids found in *Epichloë*-infected-grass associations, ergovaline is the most abundant and is considered the most potent towards mammals (Rowan et al. 1990; Rottinghaus et al. 1991; Oliver et al. 1994; Bush et al. 1997; Klotz 2015). This review will critically evaluate the data available to determine whether (a) ergovaline producing endophyte strains can be advantageous to the pastoral industries, and (b) if so, is there a threshold level below which it is safe when in pastures fed to ruminants. The focus will be on the use of *Epichloë* endophytes in perennial ryegrass and the data collected in New Zealand studies. However, endophyte-infected tall fescue (*F. arundinacea*) has been much more extensively studied especially in the USA as ergot alkaloids have long been

recognised to severely affect animal health. This research will therefore also be incorporated into this review as the experience of overseas groups will aid our interpretation of New Zealand results.

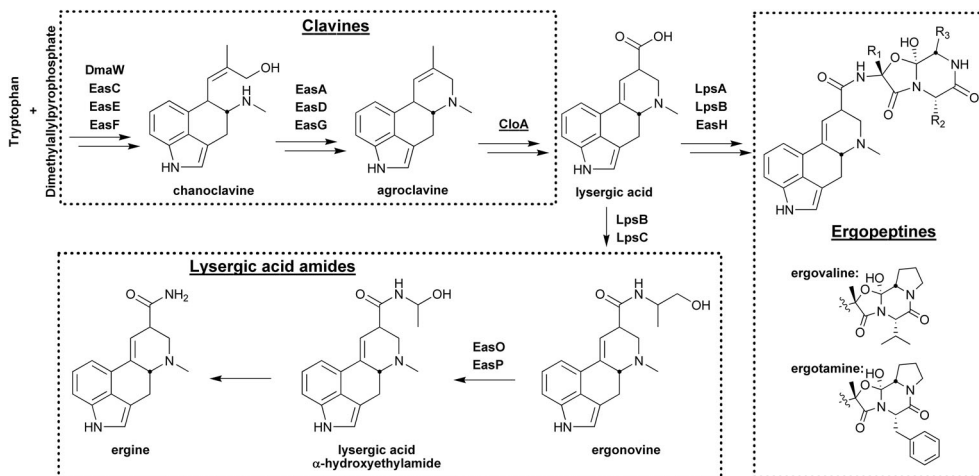
## Ergovaline in New Zealand pastures

### Ergot alkaloids

Ergot alkaloids are a group of nitrogenous compounds derived from L-tryptophan. These mycotoxins are mainly produced by *Claviceps* species (over 80 compounds) but also by other fungi and higher plants (Schiff 2006). The ergot alkaloid pathway (Figure 1) associated with *Epichloë* endophytes is well researched and understood (Porter et al. 1981; Garner et al. 1993; Schardl et al. 2006, 2009; Guerre 2015; Young et al. 2015). Ergot alkaloids can be divided into four groups based on their chemical structure: clavines (e.g. chanoclavine, agroclavine), lysergic acid, lysergic acid amides (e.g. ergonovine, ergine) and ergopeptines (e.g. ergovaline, ergotamine, ergocornine, ergocristine, ergosine, ergocryptine) (Schardl et al. 2006; Gerhards et al. 2014).

Ergovaline is the most prevalent and toxic ergot alkaloid in endophyte-infected grasses and is therefore the most widely studied and understood (Lyons et al. 1986; Schardl et al. 2009).

Ergovaline has three stereoisomers (ergovaline, ergovalinine and aci-ergovaline) and two derivatives (dehydroergovaline and dehydroergovalinine) (Lane 1999). However, ergovaline is not the only ergot alkaloid produced by *Epichloë* endophytes in perennial ryegrass and tall fescue (Lane et al. 1999; Durringer et al. 2007), and there is evidence that some of these other compounds may contribute to the animal toxicosis often attributed to ergovaline (Gadberry et al. 2003; Durringer et al. 2007). Other ergot alkaloids, including



**Figure 1.** Biosynthesis of ergot alkaloids associated with endophyte–plant symbioses. Double arrows indicate one or more omitted intermediates. Relevant enzymes associated with catalysis are indicated. At the branch point, combinations of peptide synthetases LpsA, LpsB, and LpsC are required to produce ergot alkaloids or lysergic acid amides (Lorenz et al. 2009; Ortel & Keller 2009). For the ergopeptines, R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> refer to the side-groups of the three amino acids linked to lysergic acid to create the various final products.

ergotamine, are found at very low levels in *Epichloë*-infected ryegrass using HPLC analysis (Rowan et al. 1990) and also when using mass spectrometry (Rowan and Shaw 1987), while low concentrations of ergosine, ergonine, ergocryptine, ergocryptine and ergocorinine have also been detected (Lyons et al. 1986; Schardl et al. 2006).

Lysergic acid amides, including ergine and ergonovine, are produced late in the ergovaline pathway (Fleetwood et al. 2007) and ergine has been detected in grasses infected with some *Epichloë* endophytes (Lane et al. 1999) (Figure 1). Lysergic acid amides have also been found in *Epichloë*-infected sleepygrass (*Stipa robusta*) in the southwestern USA (Petroski et al. 1992), and in drunken horse grass (*Achnatherum inebrians*) from China at levels of 2900 µg/g DM (Miles et al. 1996). Lysergic acid amides can induce stupor in grazing animals (Lane 1999).

Clavines such as chanoclavine have also been identified in *Epichloë*-infected ryegrass and tall fescue (Porter et al. 1981; Lane et al. 1999; Fleetwood 2007) (Figure 1). Clavines are derived from dimethylallyl tryptophan (DMAT), an early product in the ergovaline pathway (Panaccione et al. 2001). Chanoclavine is devoid of any ergot-like animal toxicity (Berde and Schild 1978) and has been shown to be non-toxic to mice even at high concentrations (Finch et al. 2019). Since ergot-alkaloids of the clavine class have been shown to deter the feeding of fall armyworm (*Spodoptera frugiperda*) (Clay and Cheplick 1989) they are considered worthy of further investigation for biological activity against insects (Lane et al. 1999; Schardl et al. 2009).

### Measurement of ergovaline—sampling and methods of analysis

The methods of sampling herbage for ergovaline analysis are of critical importance because of the uneven distribution of ergovaline within and between plants. This is further described in the next section, but in summary ergovaline is most highly concentrated in the crown, lower part of the leaf tiller (pseudostem) and in flower heads (Table 1). Therefore, sampling height is critical, along with the stage of reproductive maturity (Rottinghaus et al. 1991; Davies et al. 1993; Blackwell and Keogh 1999). Variation

**Table 1.** Ergovaline concentrations (µg/g DW) in different plant parts of ryegrass: (a) standard endophyte grown in the field in the Manawatu, and harvested in late spring (November 1993) (Study 1 from Lane et al. 1997a); (b) cv. 'Grasslands Nui' infected with strain AR42 (Lp19), noted as a low ergovaline expressing strain (Study 2 from Spiering et al. 2002); (c) strain 187BB showing extremely high ergovaline levels (Study 3 from Lane et al. 1997c); (d) standard endophyte from a 3 to 4-year-old pasture in Northland harvested from mid-spring to mid-autumn (Study 4 from Blackwell and Keogh 1999); (e) total herbage analysis of 'Grasslands Nui' with standard endophyte grown in Southland and harvested throughout the year (Study 5 from Rowan et al. 1990).

Plant part	Study 1	Study 2	Study 3	Study 4	Study 5
Seed	nm	nm	65	nm	nm
Inflorescence	4.2	nm	nm	nm	nm
Flowering stem	0.5	nm	nm	nm	nm
Leaf sheath	nm	0.1 to 2.0	nm	0.3 to 1.3	nm
Leaf blade	0.2	0.1	nm	0.15 to 0.4	nm
Pseudostem/stem apex	0.8	2.1	63	nm	nm
Crown/stem	3.5	3.9	38	nm	nm
Root	0.5	nm	nm	nm	nm
Total herbage	nm	nm	27	nm	0.14 to 0.83

Note: nm: not measured.

between individual tillers can exceed the level of variation observed between individual plants (Mace et al. 2014), therefore sampling needs to account for this variation also.

A further complication is determining not just the concentration in the plant as a whole, but more particularly the concentration in the parts of the plant likely to be consumed by a grazing ruminant. This in itself can be variable and is determined by a combination of stocking rate and the amount of feed available. Therefore, sampling to a standard height at or above ground level is important for developing a time series to follow changes in ergovaline concentrations but may not necessarily reflect the amount consumed by a grazing animal. Unfortunately, some studies measuring ergovaline concentrations have not recorded height of harvest above ground.

After sampling it is important to have samples frozen and dried as soon as possible and subsequently stored at low temperatures ( $\leq -20^{\circ}\text{C}$ ) or else ergovaline concentrations will decline over the first 24-hours post-harvest (Lea et al. 2014). In addition, UV light and heat can reduce ergovaline concentrations within hours (Kaur et al. 2014; Lea et al. 2014). In the appropriate low temperature conditions (e.g.  $-20^{\circ}\text{C}$ ) samples may be stored until analysed with little deterioration of alkaloids (Lea et al. 2014). Solvent, particle size, and time used for extraction also has a significant impact on efficiency of extraction (Ji et al. 2014).

In ryegrass conserved for silage or hay, ergovaline concentrations decline the longer herbage is field dried or stored (Fletcher 2005; Hume et al. 2007a). Consistent with this, a two-year study in the USA showed that ergovaline concentrations in stockpiled herbage of Kentucky 31 tall fescue infected with standard endophyte decreased by approximately 85% between December and March each year (Kallenbach et al. 2003).

Ergopeptine alkaloids were first identified in perennial ryegrass grown in New Zealand by Rowan and Shaw (1987). Procedures for ergot alkaloid analysis commonly comprise liquid chromatography with tandem mass spectrometry (LC-MS/MS) and liquid chromatography with fluorescence detection (LC-FLD) (Shelby and Kelley 1992; Garner et al. 1993; Ma et al. 1993; Porter 1995; Rasmussen et al. 2012; Crews 2015). It is acknowledged that while ergovaline is the biologically active form of the ergopeptine compounds, isomerisation with its 8-epimer, ergovalinine, can take place variably in the plant, in stored samples, and during analysis (Lane 1999). Ergovaline concentrations in perennial ryegrass herbage grown and analysed in New Zealand are often between 0.3 and 1  $\mu\text{g/g}$  DM (Easton et al. 1996), which is about 60% of the concentrations found in USA tall fescue pastures (Lyons et al. 1986; Garner et al. 1993). In New Zealand, the accepted methodology has been to measure ergovaline as the sum of both isomers (e.g. Easton et al. 1996) whereas this is not the case in the USA. Therefore, Lane (1999) postulated that the ergovaline values reported from New Zealand studies may be 30%–40% higher than comparative measurements made in the USA. This fact should be considered when comparing ergovaline concentrations reported from studies originating in the USA and New Zealand.

Urinary ergot alkaloid excretion (creatinine) has been used, in preference to serum prolactin levels, for health assessment of steers grazing endophytic pastures, where a significant negative relationship ( $r^2 = 0.86$ ) between alkaloid excretion and average daily weight gain has been established (Hill et al. 2000). ELISA technology has also been developed to measure lysergol compounds in animal urine (Garthwaite et al. 1994) and this includes all the ergot alkaloids, so this assay gives a linear response to the amount of ergovaline ingested.

### **Distribution of ergovaline in the plant**

Field grown perennial ryegrass diploids cv. 'Grasslands Marsden' and 'Grasslands Samson', along with their respective tetraploids, cv. 'Grasslands Greenstone' and the selection '4N', together with a third diploid, cv. 'Yatsyn' were sampled in April 1998 and February 1999. This showed ergovaline concentrations of below 0.3 µg/g DM in leaf lamina, but in pseudostems levels varied from between 0.6 µg/g DM ('Greenstone') and 1.6 µg/g DM ('Yatsyn') in 1998 and between 1.7 µg/g DM ('Samson') and 2.8 µg/g DM (4N) in 1999 (Popay et al. 2003). 'Marsden' and 'Greenstone' are long-rotation hybrid ryegrasses (*L. boucheanum* syn. *L. hybridum*) while 'Samson', '4N' and 'Yatsyn' are perennial ryegrasses (*L. perenne*). All were infected with standard endophyte except 'Greenstone' which was infected with a selected endophyte which produces ergovaline and peramine but not lolitrem B.

Ergovaline was monitored in endophyte-infected perennial ryegrass 'Samson' over a three-year period in southern France which showed that concentrations were strongly influenced by the stage of maturity of the plant with maximum concentrations measured at the fully ripe stage (Repussard et al. 2014). In this study ergovaline levels were similar in the tiller base and leaf blade ranging from 0.014 to 0.362 µg/g DM in the base of the plant, and 0.008–0.366 µg/g DM in the leaf blade, but were highest in the seed head/ flowering stem with levels ranging from 0.042 to 6.241 µg/g DM. In New Zealand, ergovaline concentrations have been shown to be not only highest in the flowering stem and seed-head, but also high in the basal sheath of the plant (Table 1). Heat stress symptoms are more severe in lambs forced to graze lower into the sward (Bluett et al. 2001a), due to the presence of higher levels of ergovaline, particularly in this case at ambient temperatures of 26°C–28°C. Davies et al. (1993) was able to show in ryegrass containing standard endophyte grown in Canterbury that ergovaline levels in the lower pseudostem were 1.4 and 1.5 µg/g DM (in March and April, respectively) compared with 0.3 and 0.2 µg/g DM in the first leaf. Others have also found ergovaline higher in the basal regions of both the tiller and sheath than in the lamina of perennial ryegrass and tall fescue (Lyons et al. 1986; Rottinhaus et al. 1991; Azevedo et al. 1993; Keogh et al. 1996; Lane et al. 1997a; Watson et al. 1999; Spiering et al. 2002), and almost absent in dead leaf material (Lowe et al. 2008). Ergovaline also occurs at biologically significant levels (over 1 µg/g DM when plants are fertilised with N, P and K) in the roots of tall fescue (Azevedo et al. 1993).

### **Level of ergovaline expression in New Zealand pastures**

Ergovaline expression is influenced by plant host genetics, *Epichloë* strain genetics, the interaction between host and *Epichloë* strain genetics, and the environment in which the plant is being grown.

### **Plant species effects on ergovaline expression**

The strain of *Epichloë* endophyte most commonly credited with causing ergovaline expression in perennial ryegrass has been referred to by a number of different terms including 'standard', 'wild-type', 'toxic' and 'common' endophyte. In this paper it will be referred to as standard endophyte. This endophyte was first identified in the late

eighteenth Century (Persoon 1798) and further characterised in the 1930s (Sampson 1933). It was probably introduced to New Zealand by European colonists in the nineteenth century. However, it was not until the 1980s that the alkaloid expression of the standard endophyte was elucidated to show that it produced at least three alkaloids—peramine, lolitrem B and ergovaline (Easton et al. 2001). Tall fescue with a toxic endophyte strain occurs along roadsides in New Zealand (Cunningham 1948; Wilson 1975).

Although ergovaline is found in both *Epichloë*-infected perennial ryegrass and tall fescue (TePaske et al. 1993; Gadberry et al. 2003) signs of ergovaline toxicity are rarely reported in livestock fed endophyte-infected ryegrass (Easton et al. 2001). This could be due to the co-occurrence of lolitrem B which may mask the impact of ergot-alkaloids. It has been suggested in the literature that ‘the toxic threshold of ergovaline was lower in endophyte-infected ryegrass than in endophyte-infected tall fescue’ (Guerre 2015). However, we believe that the original data was misinterpreted. In these studies, lactating ewes fed endophyte-infected ryegrass hay containing 851 µg/kg DM of ergovaline for 28 days suffered no ill effects whereas, under the same experimental conditions, lactating ewes fed endophyte-infected tall fescue hay containing 497 µg/kg DM ergovaline showed adverse effects. This therefore suggests that the toxic threshold of ergovaline is actually lower in endophyte-infected tall fescue rather than endophyte-infected ryegrass (Gadberry et al. 2003; Zbib et al. 2014b, 2015).

In tall fescue forage, ergovaline can make up 80% of the ergot alkaloids (Belesky et al. 1988) and in seed 50% (Guerre 2015) although this can be variable and much lower percentages have been reported. (Gadberry et al. 2003; Dillard et al. 2019). In perennial ryegrass herbage ergovaline may make up 70% of the ergot alkaloids (W.J. Mace unpublished data) but again this can be variable. It has been proposed that the array of ergot alkaloids (Figure 1), in particular ergopeptides found in *Epichloë* infected ryegrass, maybe less varied than in infected tall fescue, although the biological significance of non-peptide ergot alkaloids, such as ergine and chanoclavine-I, cannot be dismissed (Lane et al. 1999). In this study they found that dehydroergovaline occurred in immature inflorescences of tall fescue at about 41% to 85% of the concentrations of ergovaline, but was not present in ryegrass infected with endophyte. Shelby et al. (1997) reported that for tall fescue, in addition to ergovaline, and the known ergopeptides, ergosine and ergonine (as minor components), two unusual modified ergopeptides, dehydroergovaline and aci-ergovaline were present (Table 2). They suggested that dehydroergovaline and aci-ergovaline may be derived from the modification of ergovaline by plant enzymes rather than being direct products of the endophyte itself. These plant enzymes may be lacking in perennial ryegrass as dehydroergovaline has not been detected in this association leading to the suggestion that the difference in toxicity between *Epichloë*-infected ryegrass and tall fescue may be due, at least in part, to differences in the chemistry of the associations.

**Table 2.** Mean total (µg/g DM) and proportional ergot alkaloids in three plant parts of tall fescue measured using HPLC analysis (derived from Shelby et al 1997).

Plant part	Total ergot alkaloids	Proportion of total ergot alkaloids				
		Ergine	Ergovaline	Aci-ergovaline	Didehydroergovaline	Ergosine + ergonine
Seed	6.32	0.35	0.37	0.19	0.02	0.07
Culm	1.30	0.46	0.51	0	0	0.04
Plant	0.51	0.48	0.43	0	0	0.10



Ergine, a lysergic acid amide, has been reported in *Epichloë*-infected tall fescue seed (Table 2) at similar concentrations to that of ergovaline (TePaske et al. 1993; Shelby et al. 1997; Gadberry et al. 2003). In contrast, ergine has been tentatively identified in *Epichloë*-infected perennial ryegrass seed but at only 7% of the concentration of ergovaline (TePaske et al. 1993; Gadberry et al. 2003). Ergine has also been identified in New Zealand *Epichloë*-infected perennial ryegrass leaf material at on average about the same levels as that of ergovaline, ranging from 0.14 to 12.65 µg/kg DM across 25 genotypes infected with the same endophyte strain (Mace et al. 2014). Other lysergic acid amides, if they occur at all, are at very low concentrations in perennial ryegrass herbage (Ball et al. 1997).

Clavine alkaloids (Figure 1), including chanoclavine-I, have been identified in *E. coenophiala* in culture (Porter et al. 1981), and subsequently *in planta* in tall fescue (Lyons et al. 1986) and ryegrass (Mace et al. 2014), and in seed of endophyte-infected tall fescue and perennial ryegrass of New Zealand origin (Lane et al. 1999).

### **Epichloë genetic strain effects on ergovaline expression**

Alkaloids can be independently regulated and are controlled by both plant and endophyte genotype (Roylance et al. 1994). There is a tendency for asexual, seed-transmitted *Epichloë* endophyte strains infected into ryegrass to express higher levels (between 2.3 and 5.4 µg/g DM) of ergovaline compared with horizontally transmitted sexual endophytes (e.g. *Epichloë typhina*) that can express zero ergovaline (Siegel et al. 1990). A strong positive relationship ( $r^2 = 0.73$ ) between levels of lolitrem B and ergovaline has been observed across 459 ryegrass straw samples in the USA (Hovermale and Craig 2001). This study indicated that when 2 µg/g DM of lolitrem B is reached, approximately 0.175 µg/g DM ergovaline is present suggesting that the first clinical sign observed when animals ingest endophyte-positive perennial ryegrass will be staggers. This of course begs the question of what happens when ryegrass containing a lolitrem B free but ergovaline producing strain is ingested?

Although the standard endophyte in perennial ryegrass is characterised by the presence of ergovaline, lolitrem B and peramine, an early screen of 23 *Epichloë* strains from different populations of perennial ryegrass, predominately from Europe (Christensen et al. 1993), found that 9% expressed lolitrem B and peramine but no ergovaline, 26% had peramine and ergovaline but no lolitrem B, 48% had only lolitrem B and ergovaline, and 17% expressed none of these three alkaloids. This allowed the characterisation (in terms of genetic diversity and chemical profiles) of strains that express only ergovaline and not lolitrem B such as AR6 (also known as 187BB) (Easton et al. 2001; Fletcher 2012), and AR5 (originally marketed as 'Endosafe' (Thom et al. 2012) but now marketed as 'Endo5') (Popay and Hume 2011). Other characterised strains which express ergovaline, such as NEA2, have been commercialised (Stewart et al. 2014). While this endophyte brand is a mix of NEA2 and NEA6 it is promoted as expressing only low levels of lolitrem B and ergovaline they have the potential to produce toxic concentrations under adverse environmental conditions and have reached 0.9 µg/g DM of ergovaline in autumn (Fletcher 2012; Fletcher et al. 2017).

Some of these characterised *Epichloë* endophyte strains associated with ryegrass, such as AR5, AR6 and some NEA brand endophytes, that either have been or are commercially

available, can produce ergovaline concentrations similar to those found in ryegrass with its standard endophyte and depending on host genetics can reach 1.1 µg/g DM (van Zijl de Jong et al. 2008; Logan et al. 2015). This can result in heat stress responses similar to that of the standard endophyte (Fletcher et al. 2017).

### ***Interactions between ryegrass host and Epichloë genetics on ergovaline expression***

Plant host and endophyte strain, and interactions between these factors, can modify the quantity and type of alkaloids produced (Agee and Hill 1994; Latch 1994; Ball et al. 1995; Adcock et al. 1997; Easton et al. 2002). This variation can occur at both genotype and cultivar level. A 10-fold range of ergovaline concentration was observed amongst 19 genotypes from the same cultivar of ryegrass infected with the same endophyte (Latch 1994). Latch in the same study also noted that when endophyte strain 187BB (also known as AR6) was inoculated into the diploid cultivar 'Grasslands Pacific', the expression of ergovaline was much higher than when inoculated into 'Grasslands Greenstone', an autotetraploid hybrid ryegrass cultivar. This was most apparent during flowering under warm ambient conditions, or with regrowth soon after the harvest of seed crops (Tapper and Latch 1999). As a result, cv. 'Grasslands Pacific' Endosafe was withdrawn from the market, but cv. 'Grasslands Greenstone' continued to be marketed (Fletcher 2012). Davies et al. (1993) also noted large variations in ergovaline content with strain 187BB inoculated into 'Nui', 'Ruanui' and 'Greenstone'. In 'Greenstone', ergovaline expression was only 1.1 and 1.4 µg/g DM (in March and April, respectively) but in 'Nui' and 'Ruanui' the average ergovaline expression level was 4.8 and 3.9 µg/g DM, respectively.

As has been shown for tall fescue (Hill et al. 1991; Hill 1993; Agee and Hill 1994; Roylance et al. 1994; Adcock et al. 1997; Hiatt and Hill 1997), the host ryegrass genome has significant control over the concentrations of ergopeptides produced by the endophyte in herbage (Easton et al. 2002). This study also showed that parent-progeny correlation coefficients were high and narrow-sense heritability was estimated as 0.70 for ergovaline. It was also estimated that 41% of the genetically controlled variation in ergovaline concentrations in ryegrass was a function of mycelial mass.

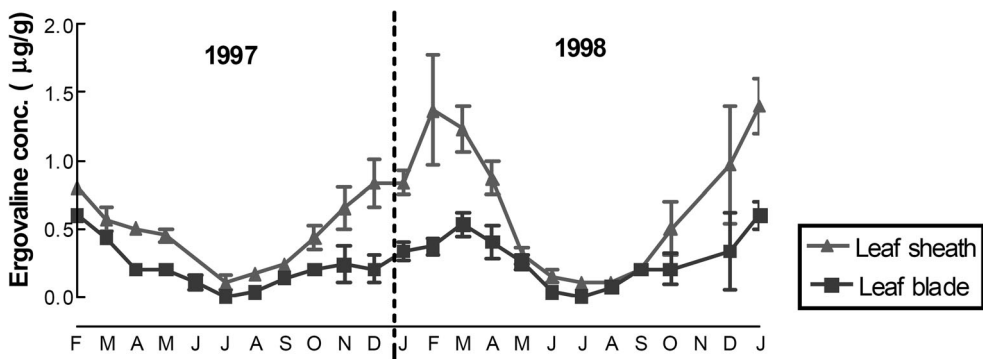
Therefore, it is not unexpected that the level of ergovaline can be low in one plant and high in another, such that when one strain of endophyte was inoculated into 19 endophyte-free Nui seedlings the ergovaline expression ranged from 3 to 27 µg/g DM among plants (Tapper and Latch 1999). Similarly, it has been demonstrated that by phenotypic selection, the level of ergovaline in tall fescue breeding lines infected with standard endophyte *E. coenophiala* can be reduced in progeny (Agee and Hill 1994). The variation in ergovaline between ryegrass plants led both Rowan et al. (1990) and Easton et al. (2002) to suggest that it may be possible to select endophyte strains for high or low levels of ergovaline. This has been the strategy behind the release of some of the NEA branded endophytes (Eady et al. 2017). While this may be partly correct, it is now accepted that the level of ergovaline expression can be driven by the host plant genetics (Easton et al. 2002) and also by environment (e.g. Barker et al. 1993; Repussard et al. 2014; Dillard et al. 2019). Selection for ergovaline levels in tall fescue is also highly heritable (Adcock et al. 1997; Hill et al. 2002). In contrast to other studies, ploidy and ryegrass species had no effect

on ergovaline concentrations in the lamina or pseudostem in a field study in Taranaki (Popay et al. 2003).

### **Environmental effects on ergovaline expression**

Several biotic and abiotic variables including season, can impact on the level of ergovaline expression. The ergovaline concentration of an endophytic ryegrass pasture in southern France varied considerably from 0.526 to 2.322  $\mu\text{g/g}$  DM over three years suggesting that abiotic factors play a key role in the expression of ergovaline levels in endophyte-infected perennial ryegrass (Repussard et al. 2014). The highest concentrations of ergovaline were observed in spring during ryegrass anthesis (Easton et al. 1996), under high summer temperatures (McCulley et al. 2014; Fuchs et al. 2017) with a further peak in the autumn (Fletcher et al. 1994; Easton et al. 1996; Fletcher et al. 2001; Bluett et al. 2005; Moate et al. 2012; Thom et al. 2013), particularly after defoliation. Blackwell and Keogh (1999) in an on-farm study in Northland found that ergovaline concentrations were higher in summer and in autumn than in spring. A similar trend was shown over a two-year period in the Manawatu (Figure 2) and in Australia (Woodburn et al. 1993) but in Southland, the highest levels of ergovaline were noted in late spring (Eerens et al. 1998).

However, even within a season ergovaline levels in pastures can vary greatly, and unpredictably. Easton et al. (1996) sampled 39 sites on 18 farms in Northland between January and April 1995. While the mean did not vary greatly throughout the season, the percentage of pastures with ergovaline concentrations above 1  $\mu\text{g/g}$  DM was 0% in March, 30% in February, and between 15% and 20% in January and April. They concluded that in seasons that combine adequate soil moisture with warm temperatures and high levels of mineralised (or fertiliser) nitrogen (Lyons et al. 1986), the biological activity of the endophyte remained high and this was reflected in the high ergovaline concentrations observed. Hume et al. (2016) reviewed literature to date on changes in endophyte and



**Figure 2.** Mean ( $\pm$  SEM) ergovaline concentrations in ryegrass leaf blade and leaf sheath components of the standard endophyte infected pasture, grown in the Manawatu region, during 1997 and 1998 (taken from Watson et al. 1999). Average maximum daily temperature for summer (December to February) in each year was 22.3°C and 22.8°C, respectively; for autumn (March to May) 18.2°C and 19.9°C; for winter (June to August) 12.7°C and 13.8°C; and for spring (September to November) 16°C and 17.3°C. Data provided by the Meteorological Service of New Zealand Ltd..

alkaloid concentrations in response to experimental warming and CO<sub>2</sub> increase, and concluded that warming resulted in increased endophyte prevalence and concomitant increase in alkaloid concentration. Plants suffering from water deficit have elevated ergovaline levels (Lane et al. 1997b), which in some studies have resulted in a doubling of the ergovaline concentration (Barker et al. 1993).

Impacts of N supply on ergovaline levels have been variable. Ergovaline concentrations can be elevated by N fertilisation (Lyons et al. 1986; Rottinghaus et al. 1991; Lane et al. 1997b; Reed et al. 2011; Repussard et al. 2014), ambient temperature and drought (Archavaleta et al. 1992; Barker et al. 1993; Eerens 1996). Fletcher et al. (2001) showed that 40% to 50% of the variation in ergovaline levels in ryegrass could be explained by soil temperature (at 1 m depth). Hunt et al. (2005) showed a negative correlation between the production of ergovaline and N fertilisation at ambient CO<sub>2</sub>, but a positive effect at elevated CO<sub>2</sub>, when coupled with high N. They proposed that this seemingly peculiar result could be related to the requirement for carbohydrates, as well as N, in alkaloid biosynthesis. However, Belesky et al. (1988) and Rasmussen et al. (2007) showed no N fertilisation effect on ergovaline concentrations while Azevedo et al. (1993) showed elevated ergovaline levels in leaf blade, stem, crown and roots of tall fescue when fertilised with N, P and K.

Even in Ireland with its mild climate it has been noted that an awareness is required of the potential adverse effect of ergot alkaloids from perennial ryegrass infected with endophytic fungi on animal health and performance (Canty et al. 2014).

## Ergovaline effects on pest resistance

Ergovaline is known to deter adult Argentine stem weevil (ASW) (*Listronotus bonariensis*) (Popay et al. 1990; Popay and Wyatt 1995). However, the benefits of this bioactivity are reduced by the fact that it is the larvae of ASW that causes greater damage to pasture (Kelsey 1958) and Popay and Lane in unpublished work (quoted in Popay and Ball 1998) found that ergovaline had no effect on ASW larval growth or development. It may, however, have a role in reducing oviposition in the pseudostem (A.J. Popay unpublished data). Some other ergot alkaloids can affect ASW with ergocryptine deterring feeding by weevil adults and larvae, and ergotamine affecting adult feeding (Dymock et al. 1989).

Ergovaline and other ergot alkaloids (ergovalinine, ergotamine,  $\alpha$ -ergosine, and  $\alpha$ -ergocryptine) are described as having strong anti-feeding activity to adult African black beetle (*Heteronychus arator*) (Ball et al. 1997; Wheatley et al. 2003; Hume et al. 2007b) compared with the simpler lysergic acid amides and clavines (Prestidge and Ball 1993; Ball et al. 1997). Ball et al. (1997) established that the ergopeptide concentration threshold required for significant black beetle feeding deterrence lay between 1.0 and 5.0  $\mu\text{g/g DM}$ , which is within the ergovaline concentration range encountered *in planta* at certain times of the year (Rowan et al. 1990; Davies et al. 1993; Fletcher et al. 1994; Ball et al. 1995; Easton et al. 1996). Interestingly, when Fleetwood et al. (2007) deleted an endophyte gene responsible for the synthesis of ergovaline, resulting in the elimination of ergovaline and lysergic acid amides, and the accumulation of lysergic acid and other clavine intermediates, feeding deterrence to African black beetle was still observed with plants infected with the mutant strain. This work therefore suggests that the clavine intermediates of ergot

alkaloids may be responsible for black beetle deterrence. Contrary to this however, clavines and simple lysergic acid derivatives were determined using a choice test as having no anti-feeding activity towards adult African black beetle, while lysergic acid amide and ergonovine had only weak anti-feeding activity (Ball et al. 1997). In this study it was the ergopeptide alkaloids ergotamine, ergovaline, ergosine and ergocryptine that provided the reduced feeding at 5 µg/g DM. Deterrence of the adult beetle by ergovaline is thought to reduce oviposition in the field leading to reductions in highly damaging root-feeding larval populations (Prestidge et al. 1994) although this tends to only delay population build-up. Siegel et al. (1989) commented that ergovaline has not been measured at biologically significant levels in root tissues, but Lane et al. (1997a) did measure ergovaline in root tissues (Table 1) although there was no measure of effects on African black beetle larval feeding in that study. Additionally, the known potent bioactive compound, loline which does get into the root system, has been shown to have a variable effect on black beetle feeding ranging from being not significant (Popay and Ball 1998) to a significant effect on larval feeding (Barker et al. 2015).

Ergovaline produced both by the standard and AR6 endophytes may be a possible factor in reducing root aphid numbers (*Aploneura lentisci*) in perennial ryegrass (Popay and Gerard 2007). Patterson et al. (1991) showed that wet weight concentrations of 5 and 10 µg/g of ergotamine deterred feeding by larvae of the Japanese beetle (*Popillia japonica*) but equivalent dry weight concentrations would be unrealistically high. In addition, ergovaline appears to have neither a deterrent nor toxic effect on either grass grub (*Costelytra zealandica*) or porina (*Wiseana* spp.), both native insects that can be destructive to ryegrass pastures in New Zealand (Popay and Ball 1998).

Despite ergovaline affecting *Pratylenchus* spp. in *in vitro* motility tests, the elimination of certain complex ergot alkaloids (ergovaline and lysergic acid amides) in one gene knockout strain, or complete elimination of ergot alkaloids in another, did not affect the ability of the endophyte to suppress populations of nematodes in *in planta* tests (Panaccione et al. 2006b).

## **Ergovaline effects on animals**

### ***Known effects of ergovaline on animals***

In general terms there are a number of important effects of ergovaline in consumed feed on animal physiology and metabolism as reviewed by Klotz (2015) and Klotz and Nicol (2016). Klotz (2015) concluded that the impacts of ergot alkaloids on livestock are not caused by the sole action of a single toxin but rather the combined impact and synergistic action of multiple ergot alkaloids. Flieger et al. (2019) further postulated that ergochrome compounds, such as secalonic acids A-C may contribute to the overall toxicity caused by ergot alkaloids.

Ergot alkaloids in feed can impact on feed intake, body temperature and respiration rate, general physiology, and overall animal production.

### ***Feed deterrent effect***

Ergovaline has a deterrent effect on grazing animals (Pennell and Rolston 2003). This has been demonstrated in cattle where a study showed animals grazing endophyte-infected tall

fescue had lower feed intakes than animals grazing endophyte-free tall fescue (Stuedemann et al. 1989; Seman et al. 1990). Similarly, another study in the USA showed that cattle fed endophyte-infected tall fescue hay, and cattle fed endophyte-free hay supplemented with pure ergotamine, had lower feed intakes than cattle fed endophyte-free tall fescue hay both at thermoneutral and elevated temperatures (Osborn et al. 1992).

This feed deterrent effect has also been observed in rats where animals fed endophyte-infected tall fescue seed had a decreased food intake under both thermoneutral and heat stress environments (Roberts et al. 2002). Furthermore, Filipov et al. (1998) showed that endophyte-infected tall fescue reduced the food consumption and weight gain of rabbits in comparison to nil endophyte controls. Later work by Panaccione et al. (2006a) using endophyte-infected perennial ryegrass with altered ergot alkaloid profiles showed that ergovaline was the compound primarily responsible for appetite suppression in rabbits. This deterrent trait has been the basis of using ergovaline expressing endophytes in turf ryegrass and fescues to deter small birds, rabbits and rodents at airports and recreational areas to avoid bird strike and situations where small animals are regarded as a nuisance (Pennell et al. 2010, 2017a, 2017b; Finch et al. 2016).

### ***Elevated body temperature and respiration rates***

Elevation of rectal temperature and respiration rate has been observed in cattle fed endophyte-infected and ergotamine diets at high temperatures although not at under thermoneutral conditions (Osborn et al. 1992). A similar effect has been observed in sheep where reduced food intakes of perennial ryegrass infected with standard endophyte have been associated with lower animal performance (Edwards et al. 1993). However, separately from effects of feed intake or environmental temperature, ergot alkaloids from fescue seed can affect the cardiovascular system of steers (Oliver et al. 2000; Eisemann et al. 2014). To counteract overheating in warm and humid conditions, the respiration rate and salivation of the animal increases in an attempt to cool itself—these are recognised as heat stress symptoms in these animals (Brookbanks et al. 1985; Easton et al. 1996).

### ***Effects on physiological systems***

Schiff (2006), Strickland et al. (2011, 2012) and Dellaflora et al. (2015) provide thorough reviews concerning the mechanisms (toxicodynamics) by which the ergot alkaloids may affect the physiological systems of animals (and humans), resulting in health and/or production deficiencies. Ergovaline has a significant effect on biogenic amines that occur naturally through the enzymatic removal of a carboxyl group (-COOH) from an amino acid. Many amines (e.g. dopamine, histamine, and serotonin) have powerful physiological effects on ruminants/mammals through their action as neurotransmitters (Purves et al. 2012). Ergot alkaloids, including ergovaline, bind these biogenic amines and stop them functioning, and as a result, can cause persistent vasoconstriction (Rhodes et al. 1991; Dyer 1993; Larson et al. 1995; Strickland et al. 1996; Larson et al. 1999; Oliver 2005; Aiken and Flythe 2014), decreased heart rate and increased blood pressure (Aiken et al. 2007, 2009). This explains why the animal's ability to regulate their core body temperature is weakened (Hemken et al. 1981; Spiers et al. 2005; Eisemann et al. 2014) and reduces blood flow to extremities, such as tails, ears and hooves (Solomons et al. 1989; Rhodes et al. 1991; McCollough et al. 1994; Oliver 1997) which can result in gangrene and death (Goodman 1952; Jensen et al. 1956; Maag and Tobiska 1956; Garner and Cornell

1978; Bush et al. 1979; Bacon et al. 1986; Thompson and Stuedemann 1993). Steers fed ergot alkaloid containing tall fescue had decreased serum concentrations of cholesterol, globulin (increased albumin/ globulin ratio), prolactin, total protein, and copper (Oliver et al. 2000).

A series of studies by Klotz with tall fescue examined the potency and efficacy of selected ergot alkaloids to induce constriction of the bovine lateral saphenous vein *in vitro* (Klotz et al. 2006, 2007, 2008, 2010). These studies showed that ergovaline is approximately 1000 times more potent (concentrations at which constriction occurs) in causing lateral saphenous veins to constrict in comparison to lysergic acid (Klotz et al. 2006, 2007, 2008). Ergot alkaloids as agonists of dopamine can reduce serum prolactin levels (Fletcher and Barrell 1984; Porter et al. 1985; Strickland et al. 1994; Fletcher et al. 1996, 1997; Oliver 1997) and in doing so can affect temperature regulation (Nicoll and Bryant 1972; Faichney and Barry 1986; Speirs et al. 1995; Fletcher et al. 1997) resulting in heat stress (hyperthermia) in warm humid conditions or hypothermia in sub-zero conditions (Craig et al. 2015). Reduced prolactin may also impair immune function (Rever 1993; Dawe et al. 1997), reduce milk yield and lower conception rates and fertility (Kramer et al. 1999; Browning 2000). Thompson et al. (1993) showed that ergovaline may be responsible for serum prolactin decline in animals grazing endophyte-infected tall fescue. It has been noted that the threshold level for suppression of prolactin by ergovaline can be less than 0.5 µg/g DM (Debessi et al. 1993; Fletcher and Easton 1997). However, unlike Strickland et al. (1994), Piper et al. (1997) showed that feeding either ergovaline (at 2.5 µg /g DM) or ergine (at 0.5 µg /g DM) to rats had no impact on serum prolactin levels. They concluded that alkaloids other than ergovaline and ergine may play a more important role in affecting feed intake, weight gains and serum prolactin.

### **Effects on animal production**

The deterrent grazing effect of ergovaline mentioned above along with reduced nutritive value, and heat stress can result in poor animal live weight production gains, milk production and growth rates (Peters et al. 1992; Watson et al. 1999; Browning 2012; Duckett et al. 2014) leading to significant costs to agriculture (Hoveland 1993).

### **Effects of ergovaline identified in New Zealand**

In New Zealand, the most obvious effect of ryegrass infected with *Epichloë* endophytes is ryegrass staggers caused by the ingesting of lolitrem B (Fletcher and Harvey 1981; Fletcher 1982). Due of the co-occurrence of lolitrem B with ergovaline in these associations it can be difficult to isolate the effects of just ergovaline. However, RH Watson (pers. comm. in a report to Meat and Wool NZ 2007) concluded that in standard endophyte pasture the presence of ergovaline is a greater factor in the reduction of feed intake and changes in diet selection behaviour in sheep than lolitrem B. By showing an increased concentration of ergovaline compared with lolitrem B in pasture over a 14 d grazing period it was deduced that sheep selected components of the pasture that did not contain ergovaline, even though they may have contained relatively high concentrations of lolitrem B, and have been nutritionally inferior to the components being avoided.

Effects of ergovaline have been identified through depressed live weight gain (Hannah et al. 1990; Fletcher et al. 1991; Fletcher and Sutherland 1993b; Browning 2012), lowered serum prolactin levels (Thompson et al. 1993; Blackwell and Keogh 1999), and increased

body temperature (Fletcher 1993b; Bluett et al. 2001a). The possibility also exists of a synergistic effect between the two toxin classes. In some New Zealand studies, there is the suggestion of ergovaline exacerbating ryegrass staggers (Fletcher and Easton 1997) while in others there has been no evidence for a synergistic effect of ergovaline to increase the effects of lolitrem B (Finch et al. 2018). In Australia, occasional severe perennial ryegrass toxicity (PRGT) is observed in sheep and Reed et al. (2016) concluded that the ingestion of ergot alkaloids combined with the impact of high solar radiation on the animals' heat load are important factors in this unusually severe form of ryegrass toxicity. The use of endophyte strains such as 187BB in perennial ryegrass (Fletcher 1993b; Easton et al. 2001) which expresses ergovaline without lolitrem B have become useful tools to understand the direct impact of ergovaline on animals under New Zealand environmental conditions. These studies, which are described in more detail in the section above reviewing 'environmental effects on ergovaline expression; indicated that ryegrass staggers may obscure the more subtle symptoms of heat stress possibly linked with the presence of ergot alkaloids. Fletcher (1993a) surmised that at ambient temperatures above 25 °C ergovaline-expressing endophytes in ryegrass may cause heat stress and result in significant losses to animal production, in both sheep and dairy cows.

Nicol and Klotz (2015) highlighted the difficulties in determining the effect of varying concentrations of ergovaline on animals due to (1) inaccurate predictions of ergovaline intake caused by the ergovaline concentration of the diet on offer not representing the concentration in the diet consumed, and (2) dry matter intake rates being ignored or difficult to estimate in field trials. The use of urine lysergol:creatinine ratios as an estimation of the amount of ergovaline consumed by animals shows some promise (Hill et al. 2000; Layton et al. 2004) at least when comparing studies conducted over time. More details on the various trials on grazing animals are presented below.

## ***Production and welfare effects of ergovaline on grazing animals***

### ***Dairy cows***

Early documented reports (Brookbanks et al. 1985) of what was then called 'bovine hyperthermia' was linked to farms in Bay of Plenty and Northland where animals were observed to have excessive salivation, inappetence, rough coats, and would stand with open mouths and protruding tongues, seeking shade and water to stand in. Although all of these farms had tall fescue present, in some instances it was only a small amount along farm fences. The condition would occur between November and April and resulted in lost production of up to 50% in one instance. Others have linked decreases in milk production in Northland to heat stress during some periods in summer and the consumption of endophyte alkaloids (Blackwell 1999).

Dairy cows in many areas of New Zealand can be affected by heat stress during summer (Easton et al. 1996; Keogh et al. 1999; Bryant et al. 2007b; deNicolo et al. 2015). Heat stress occurs when the 'heat load' of the animal is greater than its capacity to lose heat due to high ambient temperature and humidity, direct solar radiation and lack of air movement (Blackshaw and Blackshaw 1994). The combined effect of temperature and humidity can be measured through a calculated Temperature Humidity Index (THI) (Davis et al. 2003). When THI reaches 68 for Friesians and 75 for Jerseys (equivalent to 21 and 25.5 °C respectively at 75% relative humidity) New Zealand cows begin to experience the effects



of heat stress (Bryant et al. 2007b). Several overseas studies have observed milk yield reductions when the THI exceeds an average of 72 for three consecutive days even when the diet is largely made up of concentrates (Ravagnolo et al. 2000; Aharoni et al. 2002; West et al. 2003). This is related to a reduction in feed intake and a concomitant drop of around 10 g milksolids per day per unit increase in THI (Bryant et al. 2007b). Overseas studies have consistently shown that dairy cows respond to heat stress by reducing milk production (Kadzere et al. 2002; West 2003), again largely due to reduced feed intake. It is also well known that heat stress in ruminants can be exacerbated by ergovaline (Osborn et al. 1992; Aldrich et al. 1993) through causing vasoconstriction (Klotz et al. 2008, 2010). In the USA, many trials have shown negative effects of tall fescue endophyte infection on live weight gain, milk production and welfare of cattle (Hoveland et al. 1980; Hemken et al. 1981; Schmidt et al. 1982; Wallner et al. 1983; Hemken et al. 1984; Stuedemann and Hoveland 1988; Crawford et al. 1989; Hoveland 1993; Schmidt and Osborn 1993). Similarly, in Australia, ergovaline at levels of 0.4–0.75 µg/g DM in ryegrass fed as silage has resulted in a significant 4.6 L/cow/day decrease (15%) in milk production (Lean 2001). Concomitantly, milk somatic cell count increased significantly, and reproductive performance declined. Body condition score and coat condition were also negatively affected. Valentine et al. (1993) comparing cv. ‘Ellett’ perennial ryegrass with and without endophyte showed significantly lower milk yields (up to 12% lower) when cows were fed the grass with endophyte (producing both ergovaline and lolitrem B).

The importance of night temperatures was recognised by Ansell (1976) who showed that cattle during the day can accumulate heat in their bodies without adverse effects if night temperatures are sufficiently low to allow for the dissipation of heat. Fletcher (1998) therefore proposed that, if night temperatures were high, then cattle could not dissipate the heat in their bodies accumulated during the day, and it may be the impact of high night temperatures that would result in hyperthermia during prolonged hot periods.

As pointed out by Bryant et al. (2007a) in Northland, average milksolids yield is 218 kg per cow compared with Southland, where average milksolids yield is 418 kg per cow (LIC and DairyNZ 2019). Mean daily maximum air temperature is more than 5.0 °C higher in the north of the North Island (Kaitia 16.4°C) than in the south of the South Island (Gore 10.8°C) (National Institute of Weather and Atmospheric Research 2018). However, one could also argue that factors other than just temperature are impacting here, including the fact that lower levels of toxic standard endophyte, producing ergovaline, are either required or common in pastures in Southland, compared with northern New Zealand (Widdup and Ryan 1992; Eerens et al. 1998) due to different insect pest pressures.

A comparison of milk production of dairy cows grazing a ryegrass cultivar infected with the AR1 strain of endophyte (which expresses no toxins) with dairy cows grazing a standard endophyte (expressing both ergovaline and lolitrem B) through two mild summers in Northland (Ussher 2003) showed increases in milk yield of AR1 over the standard endophyte of 5% in year 1 and 1.4% in year 2. However, during the months of high temperatures (23°C–25°C average daily maximum temperatures) in January and through to mid-March the herd grazing AR1 pastures produced 21% more milk than those on standard endophyte pastures. Similarly, dairy cows grazing cv. ‘Bronsyn’ ryegrass containing AR1 in the Waikato showed a 8.9% improvement in total milksolids compared to cows grazing ‘Bronsyn’ standard-infected pastures over a 3-year period (318 versus 292 kg/cow, SED = 9.2,  $P = 0.006$ ) (Bluett et al. 2005). During this time, ergovaline levels in the

standard endophyte-infected pasture ranged between 0.17 and 0.75 µg/g DM. However, there were no significant effects of endophyte strain on mean or maximum daily cow body temperatures (using a temperature logger mounted in a progesterone-free Controlled Internal Drug Release (CIDR)) in January, February, or March. In a short-term trial, cows grazing ryegrass cultivar 'Vedette' infected with the AR1 endophyte produced 8% more milk than those grazing 'Vedette' infected with standard endophyte, and this coincided with the maximum levels of ergovaline in the standard endophyte-infected pastures (0.7 ng/ml) (Bluett et al. 2001b).

Blackwell and Keogh (1999) (also see Keogh et al. 1999) described a trial in Northland comparing two groups of 16 spring-calving, 3-year-old Holstein-Friesian cows grazing pastures either with or without ergovaline and lolitrem B. Although the presence of lolitrem B may have had an influence on this experiment they were able to establish a negative correlation between milk production of the group ingesting ergovaline and lolitrem B in January with the prevailing temperature and humidity conditions during the night, which suggest that ergovaline was having an impact. While there was no relationship between daily maximum temperature and milk production, there was a significant positive relationship ( $R^2 = 0.94$ ) between THI and milk production for cows on the endophyte free pastures, but this relationship was negative ( $R^2 = 0.60$ ) for cows on the pasture containing alkaloids. Over a milking season (1 October to 29 April) cows grazing ergovaline- and lolitrem-free pasture produced 19% more milk solids per cow (measured using herd test data) than cows grazing pastures containing these toxins. Over this period ergovaline concentrations ranged from 0.15 to 0.4 µg/g DM in the leaf blade and from 0.3 to 1.5 µg/g DM in the leaf sheath. The difference in milksolids per cow between animals grazing the two endophyte treatments was greatest between December and late March when ergovaline concentrations were highest, concentrations during this period were between 0.27 and 0.40 µg/g DM in the leaf blade and 0.8 and 1.3 µg/g DM in the leaf sheath. However, not all studies comparing standard endophyte with endophyte free pastures have shown clear and consistent differences for dairy production (Thom et al. 1997, 1999; Clark et al. 1999). Eady et al. (2017) estimated ergovaline intake by cows at three sites across New Zealand and showed that for diploid ryegrass, 'Bronsyn' with standard endophyte, ergovaline intake was 0.016–0.056 mg/kg LW<sup>0.75</sup>/day; and for diploid ryegrass 'Trojan' with NEA2, estimated ergovaline intake ranged from 0.01 to 0.029 mg/kg LW<sup>0.75</sup>/day. In their review Nicol & Klotz (2016) found only four studies showing negative effects of ergovaline intake on production (liveweight gain or milk yield) when intake was below 0.07 mg/kg LW<sup>0.75</sup>/day, all of which involved standard endophyte, with the potential confounding effect of lolitrem B expression. To allow comparison between units 0.07 mg/kg LW<sup>0.75</sup>/day is associated with an ergovaline concentration in ryegrass of 0.7 µg/g DM.

Fletcher (1998) reported dairy cows grazing standard *Epichloë*-infected ryegrass have shown unexplained lameness and swelling of the fetlock, which did not respond to antibiotics.

### **Cattle**

Easton et al. (1996) noted that there were many instances described in New Zealand where ergovaline containing ryegrass pastures resulted in cattle with high rectal temperatures, high respiration rate, panting, drooling of clear mucus from the mouth and nose, extrusion of the tongue, and searching for shade and cool. A loss of condition was reported, with rough coats often coated with mud or faeces. Production losses of up to 50% on a herd

basis were reported. To further investigate this Easton et al. (1996) sampled pastures close to ground level in the summer of 1992–1993 from Canterbury, Manawatu, Hawke's Bay and Taranaki; from pastures in Northland in the summer of 1993–1994; and from pastures in Waikato and near Dannevirke in April 1995. The results did not indicate major differences between ergovaline levels from samples taken from the different regions of New Zealand. They found that 37% of samples had ergovaline concentrations below 0.5 µg/g DM, 34% between 0.5 and 1.0 µg/g DM; 13% between 1.0 and 1.5 µg/g DM; and 16% greater than 1.5 µg/g DM. On six farms where heat stress in cattle was reported, the ergovaline concentrations ranged from 0.1 to 1.8 µg/g DM. In addition, sampling of Waikato pastures in April 1993 showed that 52% of samples had ergovaline concentrations below 0.5 µg/g DM, 30% between 0.5 and 1.0 µg/g DM; 14% between 1.0 and 1.5 µg/g DM; and 4% greater than 1.5 µg/g DM. From this Fletcher (1998) surmised that in regions prone to heat stress these ergovaline levels would be high enough in summer and autumn to exacerbate heat stress symptoms. He also noted the importance of night temperatures as discussed earlier.

Easton et al. (1993, 1996) surmised that cattle grazing standard endophyte-infected ryegrass during summer and autumn in northern New Zealand ingest herbage which may contain over 1 µg/g DM ergovaline, and sometimes over 1.5 µg/g DM. While these levels are much lower than those common in roadside tall fescue in New Zealand, they do approach the level of ergovaline found in endophyte-infected tall fescue cultivars in the USA that are well-recognised to cause elevated body temperatures and other symptoms associated with fescue toxicosis. They therefore concluded that in New Zealand, ergovaline levels in standard endophyte-infected perennial ryegrass pastures can reach levels sufficient to cause fescue toxicosis symptoms in livestock if ambient conditions are suitable, and that for many reported cases of bovine idiopathic hyperthermia, endophyte-infected perennial ryegrass pastures are a more likely cause than the very small amounts of volunteer tall fescue available to animals.

Cosgrove et al. (1996) noted that on a cool day (daily max: 17.8 °C), young cattle grazing ryegrass with an endophyte strain 187BB, which expresses ergovaline but no lolitrem B, had small but significantly elevated rectal temperatures compared with standard or nil endophyte treatments. There have been few statistically significant effects on body temperature recorded (Blackwell & Keogh 1999; Easton & Couchman 1999) despite visible heat stress being observed on-farm (a number of surveillance references are provided by Easton et al. (1996)). Given the severe effects on cattle performance in trials in USA, Lane et al. (1999) concluded that factors other than ergovaline may contribute to the toxicosis induced by endophyte-infected tall fescue. This conclusion was also reached by Gadberry et al. (2003) when comparing the effects of ergovaline alone and ergovaline plus endophytic tall fescue on lambs.

Cosgrove et al. (1996) in their study on the impact of endophyte strains on weaner cattle also noted reduced herbage intake, and diet digestibility in animals grazing ergovaline producing endophytes, but no effects on live weight gain or blood prolactin levels (Table 3). Results of this study (Cosgrove et al. 1996) indicate that ergovaline intakes of 0.048 mg/kg LW/day are required for ergovaline-related effects in cattle, at least under the environmental temperatures and relative humidity experienced in Manawatu. In contrast, with tall fescue, Debessai et al. (1993) reported reduced feed intake and reduced live weight gain in lambs at daily ergovaline intakes as low as 0.026 mg/kg LW/day.

**Table 3.** Comparison of three endophyte treatments and their effect on weaner cattle (data were derived from Cosgrove et al. 1996).

Measurement	Endophyte strain			Significance
	Standard	Nil	187BB	
Lolitre B ( $\mu\text{g/g}$ )	1.95	0	0.08	$P < 0.05$
Ergovaline ( $\mu\text{g/g}$ )	0.63	0	2.33	$P < 0.05$
Peramine ( $\mu\text{g/g}$ )	13.35	0	14.80	$P < 0.05$
Ergovaline intake (mg/day)	2.83	0	8.32	$P < 0.001$
Intake (kg DM/head/day)	3.6	4.6	3.8	$P < 0.05$
Live weight (kg)	166.5	190.5	177.5	$P < 0.05$
Diet digestibility (g/kg)	658	658	645	$P < 0.001$
Live weight gain (kg/day)	0.31	0.32	0.32	NS
Blood prolactin (ng/ml)	22.4	26.9	20.5	NS

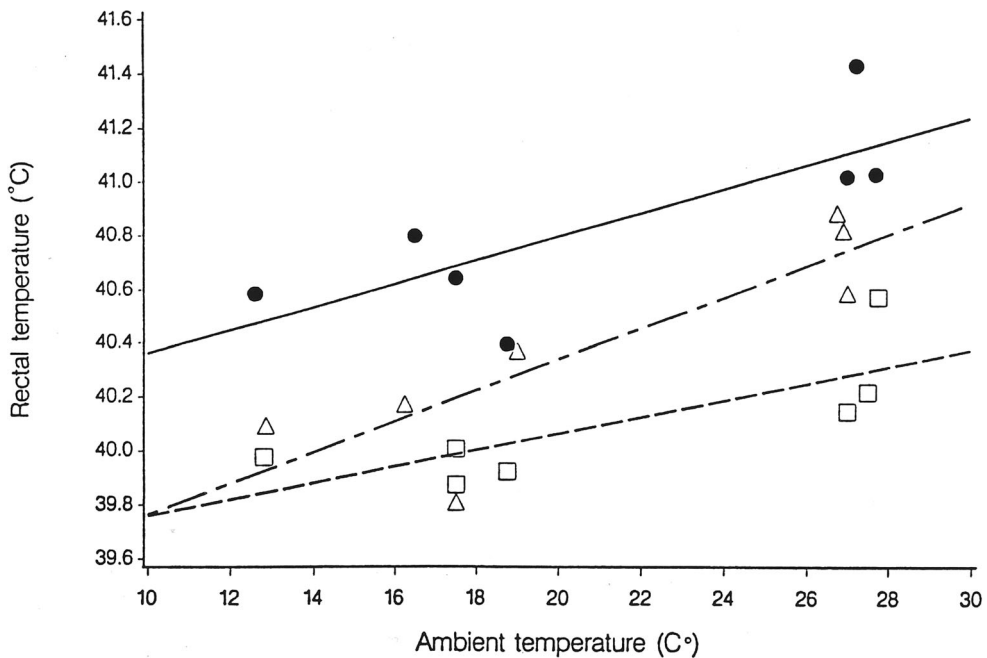
Cornell et al. (1990) showed that cattle fed herbage with up to 0.2  $\mu\text{g/g}$  DM ergovaline showed a significant increase in rectal temperature when ambient temperatures were at 31 °C or above. In noting that as little as 0.05  $\mu\text{g/g}$  DM ergovaline (fed as endophytic fescue seed) can cause measurable physiological effects in cattle, they concluded that there may be no level of ergovaline that does not cause an effect under some combinations of high ambient temperature and humidity in susceptible animals.

### Sheep

The use of diploid perennial ryegrass infected with the 187BB endophyte, expressing ergovaline but no lolitre B, has demonstrated that in New Zealand conditions, ergovaline alone can depress live weight gain, elevate body temperatures and increase respiration rates of sheep (Fletcher 1993a; Fletcher and Easton 1997). Indoor trials under thermoneutral conditions have shown that sheep fed ryegrass containing a combination of lolitre B and ergovaline have lower dry matter intakes, lower plasma prolactin and higher urine output, faecal moisture, rectal temperature and respiration rate compared to sheep fed toxin-free ryegrass (Henry et al. 2015). Although this trial utilised a diet containing both ergovaline and lolitre B, it nevertheless highlights that sheep can exhibit heat stress symptoms even under thermoneutral conditions. Heat stress, measured as increased body temperature and respiration rate, at ambient temperatures in Canterbury, New Zealand, during summer/autumn, has been reported in sheep grazing ryegrass with standard endophyte (Fletcher 1998; Fletcher et al. 1999) (Table 4). Furthermore, although rectal temperature of lambs increased with increasing ambient temperature the rectal temperature of lambs grazing ryegrass infected with the 187BB endophyte which had higher concentrations of ergovaline (and no lolitre B) were consistently higher compared to lambs grazing ryegrass infected with the standard endophyte, which had lower concentrations of ergovaline but did contain lolitre B, as well as animals grazing endophyte-free ryegrass which contained no toxins (Figure 3). At an ambient temperature of

**Table 4.** Effect of endophyte and ergovaline, in perennial ryegrass, on body temperature and respiration rate of grazing lambs, at ambient temperature of 28°C. (Taken from Fletcher 1998).

Treatment	Body temperature (°C)	Respiration rate No. breaths /minute	Ergovaline ( $\mu\text{g/g}$ )
Endophyte-free Nui ryegrass	40.1	80	0
Standard endophyte infected Nui ryegrass	40.6	111	1.8



**Figure 3.** Effect of ryegrass/endophyte association and ambient temperature, in March 1992, on rectal temperature of grazing lambs (from Fletcher 1993b). Mean ergovaline content at time of testing were 1.3  $\mu\text{g/g}$  DM for 'Nui' wild-type ( $\Delta$ - -  $\Delta$ ), 4.7  $\mu\text{g/g}$  DM for 'Nui' 187BB ( $\bullet$ - -  $\bullet$ ) and zero for 'Nui' Nil ( $\square$ - -  $\square$ ) (from Davies et al. 1993).

only 13°C the rectal temperature of the lambs grazing the high ergovaline pastures were approximately 40.55°C compared to the lambs grazing low ergovaline pastures or toxin-free pastures which had rectal temperatures of 40.05°C and 39.95°C, respectively (Fletcher 1993b). The respiration rates in this study were also observed to be higher in the animals grazing the 187BB infected pasture compared to the other two treatments. Heat stress symptoms have been observed to be more severe in lambs forced to graze lower into the sward (Bluett et al. 1999). Fletcher et al. (1999) concluded from a review of results across New Zealand that heat stress in lambs and sheep due to ergovaline would be especially prevalent in the warm humid conditions of northern New Zealand.

Fletcher et al. (1991) in a trial in Canterbury demonstrated that after 14 days lambs grazing ryegrass infected with either standard endophyte or 187BB endophyte gained less weight than lambs grazing on endophyte-free ryegrass although only the lambs grazing the standard endophyte treatment lost weight after 28 days. Fletcher (1993b) found that rectal temperatures of lambs transferred to a heat chamber indoors were highest in lambs grazing ryegrasses with high levels of ergovaline. Furthermore, ergovaline in the diet, with or without lolitrem B, increased dag weight, reduced faecal dry matter and increased fly strike of lambs (Fletcher and Sutherland 1993a). Fletcher et al. (1999) concluded that many of these symptoms resulting from ingestion of standard endophyte were similar to those described for the 'summer/autumn ill thrift' syndrome in New Zealand, resulting in stock growing at a slower than expected rate.

Aiken et al. (2011) using 187BB in perennial ryegrass showed that the auricular artery of lambs was vasoconstricted when they were exposed to ergovaline. In this study the level of ergovaline expression was 0.9–1.0 µg/g DM. The authors concluded that lambs on strain 187BB pasture were more vulnerable to heat stress than lambs grazing endophyte-free pastures. Lambs were removed from 187BB pastures after 19 days and placed on endophyte-free pastures for a further 18 days, but this was not long enough to restore the lamb's ability to physiologically adjust to increases in ambient temperature. It is generally accepted that one of the main physiological effects attributed to ergot alkaloid is a reduced ability to regulate body temperature (Oliver 1997).

It has been noted that the fertility of sheep flocks in New Zealand have been well below their potential (Knight 1990). Ewes, in New Zealand, grazing tall fescue with  $3.30 \pm 0.60$  µg/g DM (although quoted as mg/g) ergovaline had significantly lower ovulation rates and number of lambs carried to 90 days of pregnancy compared with ewes grazing nil endophyte pastures (Kramer et al. 1999). Watson et al. (1999) concluded that ewes grazing pastures where ergovaline levels exceed 2 µg/g DM are likely to suffer a reduction in reproductive performance. Exposure to ergot alkaloids in utero reduces foetal growth and muscle development in sheep (Duckett et al. 2014). This is consistent with studies on small mammals where endophyte-infected tall fescue has been found to reduce reproductive potential in rats (Varney et al. 1987, 1988) and mice (Zavos et al. 1988, 1990). Horses are particularly susceptible to ergot alkaloids resulting in increased gestation lengths, agalactia, foal and mare mortality, tough and thickened placentas, weak and dysmature foals, and reduced serum prolactin and progesterone levels (Cross 1997). Ergotamine administered to mice, rats and rabbits resulted in maternal toxicity and foetal abnormalities (Grauwiler and Schon 1973).

## Ergot alkaloids in the food chain

While the majority of studies seeking to understand the impact of ergovaline in animal feed has been focused on the direct effect on the animal itself there is another perspective to consider when animal products (milk and meat) are then consumed by humans. Although regulatory limits are set with regards to permissible levels of ergot/kg grain in the USA, Canada, New Zealand, Australia and the EU, ergot alkaloids are not regulated for food in any country (Anon 2005). However, some countries regulate ergot alkaloids in animal feeds (FAO 2004). In Canada, guideline limits for ergots in feed are 6 µg/g for pigs, 3 µg/g for dairy cattle, sheep and horses and 9 µg/g for chickens, all of which seem high. In contrast, the limit in Uruguay is much lower at 0.45 µg/g and in Oregon, the ergovaline limit for tall fescue and ryegrass straw exported to Japan as cattle feed has been set at 0.5 µg/g DM (Hume et al. 2016).

Ergovaline and lysergic acid are detectable in the urine and faeces of cattle consuming endophytic tall fescue (Stuedemann et al. 1998; Schultz et al. 2006) with concentration in rumen fluid apparently increasing over time (De Lorme et al. 2007; Ayers et al. 2009). In fact, Stuedemann et al. (1998) found that 96% of the ergopeptine alkaloids (using lysergic acid for the standard curve) consumed by cattle grazing endophyte-infested tall fescue were excreted via the urine. This led to the proposal that ergovaline is metabolised into lysergic acid that might then be absorbed through the rumen wall (Hill et al. 2001). Most of the ergot alkaloids, as weak bases, are amphipathic (i.e. possessing both polar

and nonpolar components) (Strickland et al. 2011) and their absorption would appear to be limited to the small intestine in non-ruminants (Rothlin 1933) and the fore-stomach (Hill et al. 2001) and intestine in ruminants. Ruminants appear to handle the metabolism and elimination of ergot alkaloids better than non-ruminants due to a more efficient microbial metabolism before absorption along the gastrointestinal tract (Strickland et al. 2011). Using Caco-2 cells derived from human colon carcinoma, Shappell and Smith (2005) showed that ergovaline and its naturally occurring isomer, ergovalinine, readily crossed intestinal cells intact and at similar rates.

LD50s for a number of ergot alkaloid analogues have been determined orally and intravenously (i.v.) using mice, rats and rabbits (Griffith et al. 1978). Rabbits were the most sensitive to ergot alkaloids. Ergotamine showed an intravenously administered LD50 of 265 mg/kg in mice with toxicity being 7 and 88 times greater in rats and rabbits, respectively. Not surprisingly oral toxicity, the most relevant route of administration, was considerably lower than that determined by intravenous dosing with the LD50 by gavage being 3200 mg/kg in mice, 1300 mg/kg in rats and 550 mg/kg in rabbits (Griffith et al. 1978). Symptoms associated with these acute intoxications include vomiting, diarrhoea, cardio-vascular collapse, convulsions, agony and abortions (Guggisberg 1954; Lewin 1962). Ergotamine has been fed to rats at concentrations of up to 500 mg/kg for 4 weeks. This study showed a dose-dependent decrease in food intake and weight gain with females being more susceptible than males, leading the authors to conclude that this was likely due to a decreased palatability at concentrations of >100 mg/kg ergotamine. A no-observable-adverse-effect-level (NOAEL) was set at 0.3 mg/kg/day. A further rat study was conducted using appropriate dose rates for a period of 13 weeks. Despite the extended time period, the NOAEL in this study was 0.4 mg/kg/day ergotamine. Similar 4-week studies have been conducted with ergometrine and  $\alpha$ -ergocryptine which showed similar NOAELs (0.6 and 0.3 mg/kg/day, respectively). Furthermore, developmental and reproductive toxicity, effects on lactation and genotoxicity have been investigated for some of the ergot alkaloid analogues, although none have been conducted using ergovaline (EFSA 2012). Ergotamine has therapeutic applications in humans, most commonly for the treatment of migraine headaches. The usual oral dose for this purpose is 1–2 mg ergotamine per dose with a limit of up to 6 mg/day, 12 mg/week and 20 mg/month.

The risk to human health is a function of both toxicity of the substance as well as the exposure. It is therefore important to consider the amounts of ergovaline which can be feasibly ingested by a consumer. There is no convincing evidence that ergot alkaloids accumulate in edible tissues of animals fed ergot alkaloids. Exposure of dairy cows to 50 g ergot sclerotia per animal (approximately 100 kg body weight) resulted in total ergot alkaloid concentrations in milk of 0.086 mg/litre (Parkheava 1979). In another study, dairy cows were fed an alkaloid concentration of 1835  $\mu$ g/animal/day (equivalent to 3  $\mu$ g/kg body weight) with no residues of ergot alkaloids in milk (Wolf et al. 1995). The authors surmised that the carry-over rate of ergot alkaloids into milk is less than 10% of the ingested dose. Consistent with this hypothesis, Schumann et al. (2009) were unable to detect ergot alkaloids in the milk or blood of cows fed ergot-contaminated concentrate at an alkaloid exposure rate of 4.1–16.3  $\mu$ g/kg of body weight. However, in contrast, low but detectable concentrations of ergovaline were detected in the milk of lactating ewes fed hay of perennial ryegrass infected with standard endophyte (Zbib et al. 2015). Ergovaline could also be detected in the liver and kidneys of these animals but not in

the muscle, fat or brain (Zbib et al. 2015). Also showing ergovaline residues was Realini et al. (2005) who grazed steers on endophyte-infected tall fescue and detected ergovaline in sub-cutaneous fat tissue.

Overall, the data available on ergovaline residues in animals is limited but the studies that are available point to the presence of only very low concentrations of ergovaline in milk and animal tissue. It is generally accepted that ergot alkaloids in animal tissues at these levels will not be an important source affecting humans (Anon 2005).

## Threshold concentrations for ergovaline

As detailed earlier it is difficult to accurately compare the results of different studies due to the use of different methodologies of sampling as well as pre-analysis handling and storage of samples. Comparison, and the proposal of a threshold concentration for ergovaline, is further complicated by the common co-occurrence of lolitrem B under New Zealand conditions and the suggestion that the toxic threshold is different in tall fescue and perennial ryegrass. Hume et al. (2016) has summarised the concentrations for a toxic threshold of ergovaline in animal feed for both ruminants and monogastric animals using data published by di Menna et al. (1992), Stamm et al. (1994); Tor-Agbidye et al. (2001), Aldrich-Markham et al. (2003), Fink-Gremmels (2005), Alabdouli et al. (2014), and Zbib et al. (2014a). These toxic thresholds were 0.40–0.75 µg/g DM for cattle; 0.50–0.80 µg/g DM for sheep; and 0.30–0.50 µg/g DM for horses, except for mares in late pregnancy when the safe threshold level is zero. Cornell et al. (1990) reported that even 0.05 µg/g DM ergovaline in tall fescue can cause increased body temperature in cattle. Ergovaline levels of 0.5–1.5 µg/g DM in leaf is considered comparable to those associated with symptoms of toxicity in endophyte-infected tall fescue in south east USA (Easton et al. 1993).

In New Zealand, for sheep (hoggets) fed 0.0–0.075 mg ergovaline/kg LW there was a linear decrease in liveweight gain from 110 to 60 g/d ( $P < 0.01$ ) and 30 days later, from 35 to –130 g/d ( $P < 0.05$ ); and for cattle (heifers) fed 0.0–0.035 mg ergovaline/kg LW there was a decline in liveweight gain from 1.0 to 0.85 kg/d ( $P < 0.05$ ) (Layton et al. 2004). A study conducted in New Zealand and summarised in 2011 on ‘The Beef Site’ concluded that cattle are slightly less susceptible to ergovaline toxicity than sheep with respect to live weight gain suppression (<http://www.thebeefsite.com/articles/2932/vasoconstriction-heat-stress-caused-by-ergot-alkaloids-fescue-toxins/>). They provided estimates of ergovaline intake and urine lysergol:creatinine ratios deemed to be toxic and sub-toxic for cattle and sheep (Table 5).

**Table 5.** Estimates of toxic and sub-toxic ergovaline intake levels and concentrations in pasture fed to cattle and sheep, and urine lysergol:creatinine ratios.

Measure of ergovaline exposure	Animal	Toxic threshold	Sub-toxic threshold
Pasture ergovaline concentration (µg/g DM)	Sheep	>0.9	0.03
	Cattle	>0.9	0.3
Ergovaline intake per kg of animal live weight (mg/kg liveweight <sup>0.75</sup> )	Sheep	>0.08	0.03
	Cattle	>0.11	0.03
Urine lysergol: creatinine ratio	Cattle	>7.0	<4.0
	Sheep	>4.0	<2.5

Note: Table is derived from <http://www.thebeefsite.com/articles/2932/vasoconstriction-heat-stress-caused-by-ergot-alkaloids-fescue-toxins/>



In a review seeking to understand the impact of ergovaline intake from New Zealand pastures Nicol and Klotz (2016) used studies from nine publications (including the Layton et al. 2004 study) to conclude that feed intake or animal production may be depressed when ergovaline intake is greater than 0.07 mg/kg liveweight<sup>0.75</sup>/day or when ergovaline levels in pasture were 0.7 µg/g DM or higher. They pointed out that both of these levels were much higher than thresholds suggested in the USA for animals feeding on tall fescue where ergovaline effects might occur above intakes rates of 0.035–0.045 mg/kg liveweight<sup>0.75</sup>/day (Bohnert and Merrill 2006) and ergovaline pasture levels of 0.475 µg/g DM (Stamm et al. 1994). They concluded that a high proportion of New Zealand pastures should (on average) not contain sufficient ergovaline to provide an ergovaline intake high enough to depress animal productivity or cause animal-welfare concerns. However, Fletcher et al. (1994) indicated that significantly elevated body temperature and respiration of lambs can occur with ergovaline concentrations of only 0.5 µg/g DM. He also commented that the time taken for the body temperature of affected animals to return to normal was slow and observed over a 10-day period on endophyte-free pastures the elevated body temperature of affected animals remained un-changed. He concluded that - ergovaline, even at low concentrations, affects thermo-regulation in lambs and is undesirable in perennial ryegrass-endophyte associations.

In a later report, Fletcher et al. (2017) considered that many farmers do not appreciate the potential for ergovaline to induce animal production problems especially since it is only relatively recently that endophytes which express ergovaline have been marketed. Some of these commercially-available endophytes that express ergovaline can result in increased body temperature and respiration rates similar to that which would occur in animals grazing standard endophyte-infected pastures when exposed to heat stress. However, the severity of ergovaline-induced effects in animals grazing endophyte-infected pastures will depend on farm type, location, balance of toxic and non-toxic pastures in the system, the number and age of sheep exposed to peak toxin levels, climatic conditions and management (Fletcher et al. 1999).

Animals suffering from heat stress is a welfare issue that can be avoided through providing shade and reducing the consumption of ergovaline. Other than providing endophyte-free ryegrass pastures which are unlikely to persist in most areas of New Zealand there are several non-ergovaline producing endophytes available (Fletcher 1999; Thom et al. 2007; Fletcher 2012; Caradus et al. 2013; Johnson et al. 2013; de Bonth et al. 2015) with active discovery pipelines searching for further suitable strains. The inclusion of white clover in the grazed diet has also been associated with reducing the impact of ergovaline producing endophytes on milk production (Clark et al. 1996; Fletcher 1998).

## Concluding comments

In identifying endophytes for use in ryegrass pastures in New Zealand there have been two directions pursued. Either -

1. That the minimum standard should be the exclusion of all ergopeptine alkaloids and lolitrems (Fletcher and Easton 1997); or

2. To enhance the stability of natural defences against invertebrate pests some ergopeptide presence may be beneficial (Popay and Rowan 1994; Ball et al. 1995; Popay and Wyatt 1995).

Both arguments have their strengths and weaknesses and it is a matter of balancing these to ensure the end-user has access to a product that satisfies their needs – a persistent pasture with low to nil animal toxicity in terms of both animal production and welfare. As noted above there is a significant risk in assuming that low ergovaline expressing endophyte strains will have the same low alkaloid expression irrespective of the host genetics and environment in which it is grown (Lane et al. 1997b). Endophyte strains that produce no ergovaline and yet provide good grass persistence are available, and widely used (Caradus et al. 2013). Future endophyte strains used in New Zealand should seek to avoid any ergovaline expression as one cannot assume that farmers graze swards to ensure that portions of the plant with highest ergovaline content are avoided, and that seasonal spikes in ergovaline content do not occur. In addition, it is predicted that temperatures across New Zealand, particularly in the North Island, will increase (Ministry for the Environment 2008, 2018) and this alone may exacerbate potential impacts of ergovaline on animal health and welfare.

## Disclosure statement

Grasslanz Technology Ltd is the intellectual property owner of AR1, Endo5, and AR6 (strain 187BB).

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