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# Perennial ryegrass endophyte effects on plasma prolactin concentration in dairy cows

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**Abstract** The aim of the two experiments reported here was to determine whether cows grazing perennial ryegrass infected with wild endophyte (*Neotyphodium lolii*) had reduced plasma prolactin concentrations compared with cows consuming a diet containing zero or low levels of ergovaline. The seasonal profile of plasma prolactin concentration was determined in 27 dairy cows grazing perennial ryegrass infected with wild endophyte, and in 28 cows fed a total mixed ration (TMR) diet in the 1999/2000 season. The concentration of prolactin in blood plasma from cows fed TMR was generally higher than the pasture-fed cows from November to April (20.6 versus 16.9 ng ml$^{-1}$, standard error of difference (SED) = 1.13), but the magnitude of the difference changed over time. Peak ergovaline levels of 0.8 mg kg$^{-1}$ were recorded in ryegrass pasture in mid March 2000. In a second experiment, 18 cows grazing perennial ryegrass infected with the novel endophyte AR1 (no ergovaline or lolitrem B production) had higher concentrations of prolactin in February 2001 than 18 cows grazing ryegrass infected with wild endophyte (25.2 versus 14.4 ng ml$^{-1}$, SED = 2.66), although concentrations were similar during January 2001. Prolactin concentration does not provide a reliable indicator of endophyte effects in dairy cows.

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**Keywords** ergovaline; *Lolium perenne*; milk yield; *Neotyphodium lolii*

## INTRODUCTION

Most perennial ryegrass (*Lolium perenne*) in New Zealand contains the fungal endophyte *Neotyphodium lolii*. The ryegrass/endophyte association produces many chemicals, including peramine which acts as a feeding deterrent to Argentine stem weevil (*Listronotus bonariensis*). Other alkaloids produced include lolitrem B which can cause ryegrass staggers in grazing animals and ergovaline which has been associated with increased severity of heat stress in sheep. Another response by sheep consuming ryegrass endophyte can be a reduction in prolactin concentration. Fletcher & Easton (1997) reported that as ambient temperature increased above 22°C, prolactin concentrations in sheep grazing endophyte-free ryegrass increased rapidly, while concentrations in sheep grazing endophyte-infected ryegrass remained low. Seasonal correlation between ambient temperature and prolactin concentration may reflect a role for prolactin in thermoregulation, probably through its effect on fluid balance (Faichney & Barry 1986). Prolactin is also vital for the initiation of lactation at parturition, but less important during established lactation in the dairy cow (Knight 2001).

Prolactin concentrations can be lower in steers grazing tall fescue (*Festuca arundinacea*) infected with endophyte (*Neotyphodium coenophialum)* than in those grazing endophyte-free tall fescue (Oliver et al. 2000). Ergopeptide alkaloids, mostly ergovaline, produced by this association may be responsible for inhibiting prolactin release due to their dopamine receptor activity (Larson et al. 1995, 1999). Because perennial ryegrass infected with *Neotyphodium lolii* endophyte also produces ergovaline, the work of Larson et al. (1999) could explain the reduced prolactin concentrations reported in sheep. Fletcher (1999) found that sheep grazing 'Grasslands Nui' perennial ryegrass infected with the novel endophyte AR1 (which produces no ergovaline or lolitrem B) had similar prolactin concentrations to lambs grazing 'Grasslands Nui' ryegrass without endophyte, but higher concentrations than lambs grazing 'Grasslands Nui' infected with the wild endophyte. Few data have been published for dairy cows.

The aims of this paper are to (i) report two experiments designed to test whether ryegrass endophyte reduced plasma prolactin concentrations in dairy cows, and (ii) discuss whether or not prolactin is a reliable indicator of endophyte effects in dairy cows.

## MATERIALS AND METHODS

### Experiment 1

Plasma prolactin was measured in 55 Holstein-Friesian dairy cows grazing either pasture as their sole feed source ($n = 27$) or fed a total mixed ration (TMR) based on maize silage, grass silage, whole cottonseed and concentrate ($n = 28$) (Kolver 2001). Grass silage was made from endophyte-infected perennial ryegrass pasture in October 1999 (ergovaline level of 0.4 mg $kg^{-1}$), but comprised only 20% of total dry matter (DM) intake from November to December 1999, and 37% of total DM intake from January to April 2000. Prolactin measurements were made every 3 weeks from 2 November 1999 to 20 April 2000. Grazed pastures consisted of endophyte-infected perennial ryegrass, which were sown between 1996 and 1999.

### Experiment 2

Plasma prolactin was measured in 36 Holstein-Friesian dairy cows grazing 7-ha farmlets sown with either perennial ryegrass infected with wild endophyte ($n = 18$) or perennial ryegrass infected with AR1 endophyte (no ergovaline or lolitrem B) ($n = 18$), on 3 consecutive days during each of January and February 2001. Perennial ryegrass pastures were established in autumn 2000 and contained white clover (*Trifolium repens*).

### Measurements

*Prolactin concentration in blood plasma*

Blood samples were collected from each cow by tail venipuncture into EDTA vacutainers after morning milking in Experiment 1 and before afternoon milking in Experiment 2. Blood samples were chilled immediately then centrifuged and analysed for plasma prolactin concentration by radioimmunoassay (Prolactin Antigen and Antiserum kit; National Institute of Diabetes and Digestive and Kidney Diseases, Maryland, United States) as described by Pearson et al. (1996). The inter-assay and intra-assay variations in Experiment 1 were 10.1 and 9.0% at 21 ng $ml^{-1}$, and in Experiment 2 were 2.6 and 11.0% at 25 ng $ml^{-1}$.

*Proportion of ryegrass, endophyte infection, and alkaloid concentration in pasture*

In both experiments, pre-grazing herbage samples were collected for 5 days before collection of blood samples. Ten strips of herbage were cut to ground level and bulked for each paddock. A subsample of pasture was dissected into ryegrass, other species, and dead material, before oven drying at 95°C for 36 h and weighing. The proportion of ryegrass in live herbage was then determined. A subsample of perennial ryegrass was frozen at –20°C, before freeze-drying and grinding through a 1-mm diam. sieve. This sample was analysed for ergovaline concentration using high performance liquid chromatography (HPLC) techniques (Barker et al. 1993). Endophyte infection in the paddocks grazed by cows was determined from 20 randomly collected tillers per paddock in May 2000 in Experiment 1 and in October 2000 in Experiment 2 using the tissue print-immunoblot method (Gwinn et al. 1991).

### Statistical analysis

Prolactin data from Experiment 1 were analysed at each sampling time individually using residual maximum likelihood (REML). The mean of the sampling times was calculated for each cow and was also analysed in this way. Data from Experiment 2 were analysed at each sampling time individually using analysis of variance (ANOVA).

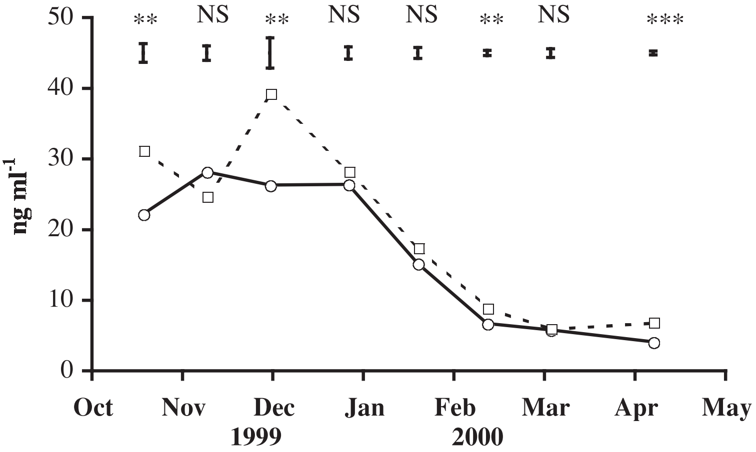


**Fig. 1** Mean seasonal profile of plasma prolactin concentration from dairy cows grazing perennial ryegrass pasture infected with endophyte ($n$ = 27) (—○—) or fed a total mixed ration diet ($n$ = 28) (---□---) from November 1999 to April 2000 (vertical bars indicate standard error of difference (SED), NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (Experiment 1).

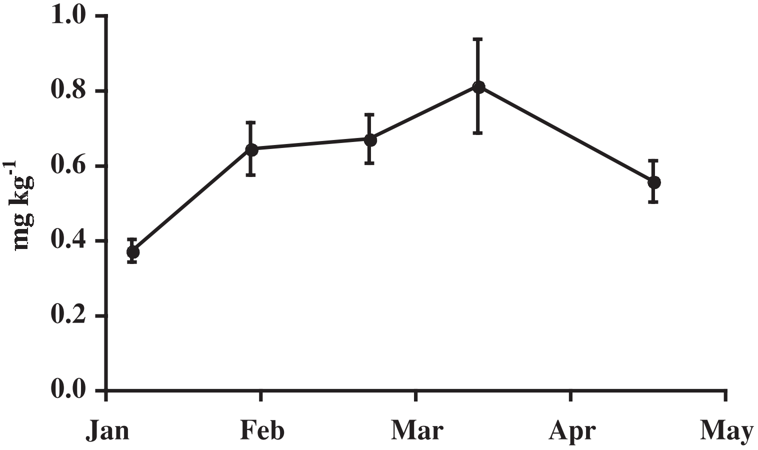


**Fig. 2** Mean ergovaline concentrations in perennial ryegrass pasture infected with endophyte rotationally grazed by dairy cows from January to April 2001 (vertical bars indicate standard deviation (SD)) (Experiment 1).

In both experiments the cow variance component was used as the experimental error. All statistical analyses were carried out using GenStat (2000).

## RESULTS AND DISCUSSION

The 3-weekly prolactin concentrations of cows grazing perennial ryegrass infected with wild endophyte compared with those fed a TMR are shown in Fig. 1 (Experiment 1). To our knowledge, this is the first report of the seasonal profile of plasma prolactin concentration for dairy cows in New Zealand. Prolactin concentrations were highest on the first measurement dates in early November to mid January, and then steadily declined to low concentrations in late February to late April 2000 (Fig. 1). Concentrations of plasma prolactin vary according to season of the year, daylength, and temperature (Hill et al. 2000). On four occasions prolactin concentrations were similar ($P > 0.05$) and on four occasions prolactin concentrations were lower in cows grazing pasture than fed the TMR diet ($P < 0.01$). Perennial ryegrass in grazed pastures accounted for most (96%) of the live herbage and it was highly infected with endophyte (98%). Peak ergovaline levels in ryegrass pastures were recorded in mid March at 0.8 mg $kg^{-1}$ (Fig. 2). Thom et al. (1999) has reported ergovaline levels ranging from 0.4 to 0.9 mg $kg^{-1}$ from pasture samples collected over 3 years (April 1994, 1995, 1996) at a nearby site. After an analysis of 23 pasture samples collected from Waikato dairy farms in April 1993, Easton et al. (1996) reported that only four exceeded ergovaline concentrations of 1 mg $kg^{-1}$. Although the TMR diet did contain silage made from endophyte-infected perennial ryegrass, it comprised only 20–37% of the diet and as a result the amount of ergovaline consumed would have been low compared with the intake of the pasture-fed cows.

Prolactin concentrations were similar between treatments in the previous season of this experiment (1998 and 1999), except during February 1999 when prolactin concentrations were lower in the cows grazing pasture (6.0 cf. 22.7 ng $ml^{-1}$; Davis et al. 2000). In a short-term experiment reported by Auldist & Thom (2000), cows grazing endophyte-infected ryegrass had similar prolactin concentrations to cows grazing endophyte-free ryegrass, although levels of ergovaline were low (<0.1 ng $ml^{-1}$) in the endophyte-infected pasture. It is well documented that sheep grazing perennial ryegrass infected with wild endophyte have lower concentrations of plasma prolactin than sheep grazing ryegrass without endophyte (Fletcher & Easton 1997). However, the effect of endophyte on prolactin concentration in this experiment with dairy cows was small and variable over time. This could be due to differences in grazing management, grazing behaviour or sensitivity to alkaloids. For example, dairy cattle are moved more frequently to fresh pastures than sheep and are therefore less likely to be forced to graze into the base of the sward where ergovaline concentrations are typically highest (Lane et al. 1997).

Current evidence also suggests that decreased prolactin levels often observed in cattle grazing endophyte-infected tall fescue in the United States (Porter & Thompson 1991) are more pronounced than differences observed in cattle grazing endophyte-infected perennial ryegrass in New Zealand. An explanation for this could be lower concentrations of ergovaline in ryegrass than in tall fescue, the existence of other unknown factors exacerbating the effects of ergovaline (Easton & Couchman 1999) or due to the presence of additional modified ergopeptides in the tall fescue association (Lane et al. 1999). For example, both the ergot alkaloids ergotamine and ergonovine present in endophyte-infected tall fescue have individually been shown to decrease prolactin concentrations in cattle (Browning & Leite-Browning 1997). The consumption of ergovaline by cows grazing ryegrass pastures infected with wild endophyte was presumably not high enough to depress serum prolactin levels on all occasions in this experiment.

In Experiment 2, prolactin concentrations in cows grazing perennial ryegrass pastures infected with wild endophyte or AR1 endophyte (no ergovaline or lolitrem B) were similar in January (Table 1). However, in February, prolactin concentrations were 43% lower in the cows grazing ryegrass infected with wild endophyte compared with cows grazing AR1-infected ryegrass. Perennial ryegrass comprised more than 85% DM of live herbage and the proportion of tillers infected with endophyte was 98% in both the AR1 and wild endophyte-infected ryegrass farmlets. In both the January and February measurement periods, ergovaline levels in samples of herbage on offer were c. 0.4 mg $kg^{-1}$ in the wild endophyte-infected pastures. To detect effects of ergovaline on prolactin in sheep, ambient temperatures must be greater than 22°C (Fletcher & Easton 1997). In this experiment, the mean ambient temperatures in January and February exceeded 22°C and were similar (27.6 and 28.1°C, respectively). These inconsistent effects of ryegrass endophyte on prolactin concentration are in agreement with those reported for tall fescue endophyte effects in cattle (Hill et al. 2000).

Prolactin concentration has been used to indicate whether sheep have consumed ergopeptide alkaloids (Fletcher & Easton 1997). However, prolactin concentration is of little value on farms as an indicator of endophyte effects because concentrations need to be compared with endophyte-free control animals at the same time (Fletcher et al. 1999). Prolactin concentration is also highly variable between animals and under different environmental conditions (Hill et al. 2000). Hill et al. (2000) noted that steers grazing endophyte-infected tall fescue usually had lower prolactin concentrations than those grazing endophyte-free tall fescue, but there were several occasions when values were similar. They also reported that the concentration of ergopeptides in urine samples were

**Table 1** Ergovaline (mg $kg^{-1}$) from pre-grazing herbage samples and plasma prolactin concentration (ng $ml^{-1}$) from cows grazing perennial ryegrass pasture infected with wild endophyte ($n = 18$) or AR1 endophyte ($n = 18$), in January and February 2001 (Experiment 2). NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; SED, standard error of difference.

| | AR1 | Wild | SED | Significance |
|---|---|---|---|---|
| January | | | | |
| Ergovaline concentration | 0.0 | 0.4 | – | – |
| Prolactin concentration | 15.5 | 13.0 | 2.01 | NS |
| February | | | | |
| Ergovaline concentration | 0.0 | 0.4 | – | – |
| Prolactin concentration | 25.2 | 14.4 | 2.66 | *** |

less variable and more dynamic than prolactin, and this could be used to determine whether cattle had consumed endophyte-infected tall fescue. The coefficients of variation for urinary alkaloid excretion were lower (46–65%) than prolactin (64–142%). Urinary alkaloid excretion diagnosis may be developed as a tool for determining whether New Zealand animals had been consuming perennial ryegrass infected with wild endophyte (L. R. Fletcher pers. comm.).

Prolactin is an anterior pituitary hormone and is best known for its action on the mammary gland, but there is evidence that it is involved in over 300 separate functions in vertebrates (Bole-Feysot et al. 1998). The secretion of prolactin at parturition stimulates subsequent milk production in dairy cows. Blocking the periparturient rise in prolactin with bromocriptine in cows has been found to lower prolactin concentrations, delay the onset of lactogenesis and reduce milk yield for a variable period postpartum (Knight 2001; Prosser & Auldist unpubl. data). Bernard et al. (1993) reported that prepartum consumption of endophyte-infected tall fescue hay reduced prolactin secretion by c. 30%, but this had no effect on the amount of milk produced. The levels of ergovaline in perennial ryegrass pasture over early spring calving would be unlikely to affect prolactin concentration because ergovaline levels tend to be low at this time (Easton 1999), but cows calving in autumn could be at greater risk, which could be the topic of further research.

Suppression of prolactin secretion usually has little effect on milk yield during established lactation in cows (Akers et al. 1981). For example, Plaut et al. (1987) reported that administering prolactin before or after peak lactation did not increase milk yield. Correlations between prolactin and milk yield in cows in established lactation are also low. In a study comparing high genetic merit with low genetic merit cows, Sorensen et al. (1998) reported no difference in prolactin concentrations, despite a 33% difference in milk yield. Knight (2001) postulated that mammary tissue has developed a high avidity for any prolactin that is present in the circulation and so can respond adequately to very low concentrations of the hormone. Furthermore, it may have acquired the means of producing its own prolactin locally within the mammary gland. Malven et al. (1987) has hypothesised that this reservoir of prolactin could be used during an emergency such as when cows have ingested compounds inhibiting prolactin release like those found in endophyte-infected tall fescue. Therefore, it is unlikely that the reduced prolactin concentration in the cows grazing wild endophyte-infected ryegrass in the current studies would have directly reduced milk yield.

## CONCLUSIONS

The effect of *Neotyphodium lolii* on prolactin concentration in these two experiments with dairy cows was small and variable over the season. Plasma prolactin concentration did not provide a good indicator of endophyte effects because of its variability. The use of plasma prolactin as an indicator of endophyte effects is also restricted to experiments that have control animals fed ergovaline-free feed under the same conditions.

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