# Endophyte and dairy production in New Zealand: experience at the Dairying Research Corporation

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## Abstract

Sixteen short term tests of the effects of ryegrass (*Lolium perenne* L.) endophyte on milk production from dairy cows were carried out at Dairying Research Corporation (DRC) sites in Taranaki (1992/93) and Waikato (1993–1999). Increasing contamination of endophyte-free areas with volunteer endophyte-infected ryegrass could have modified milk production responses to endophyte in the first of two trials in the Waikato (1993–1996). Test periods were in spring, summer and autumn. Significant effects of endophyte on milk production were detected on only 4 occasions. Small (<6%) reductions due to endophyte occurred in spring 1994, summer 1995/96 and spring 1997, and in autumn 1998 cows grazing pastures with high (H) levels of endophyte produced more milk than those grazing pastures with low (L) endophyte levels. Herbage levels of lolitrem B and ergovaline were low in spring 1994 and spring 1997 (<0.4 µg/g DM) compared with peak levels in summer of 1.2 to 3.1 µg/g DM. Ryegrass staggers occurred in cows grazing H pastures in summer 1993/94 and summer/autumn 1994/95, but not when there was an endophyte effect in summer 1995/96. Herbage ergovaline levels and ambient temperatures were generally below critical levels for development of clinical heat stress in cattle, and cow rectal temperatures were unaffected by treatments. Overall effects of endophyte on milk production were considered to be small and inconsistent.

**Keywords:** alkaloids, contamination, dairy cows, endophyte, ergovaline, lolitrem B, *Lolium perenne*, milk production, *Neotyphodium lolii*, perennial ryegrass, somatic cell counts

## Introduction

The association between endophyte and perennial ryegrass has been recognised in New Zealand for more than 50 years (Neil 1940). However, the association of the endophyte with animal diseases such as ryegrass staggers (Fletcher & Harvey 1981), the possibility of heat stress (Easton *et al.* 1996) and the protection of perennial ryegrass from insect attack (Prestidge *et al.* 1982), have been discovered only recently.

Research has identified three important alkaloids that are produced by the ryegrass endophyte: lolitrem B, ergovaline and peramine. The first two of these are toxic to animals. Lolitrem B causes ryegrass staggers and ergovaline is a cause of heat stress in warm, humid environments. Peramine is not toxic to animals but protects the plant from attack by insects such as Argentine stem weevil. The concentrations of toxins in ryegrass herbage peak in summer, often in association with water stress and high ambient temperature, increasing the chances of effects on animal health (Barker *et al.* 1993; Hawkes *et al.* 1995; Thom *et al.* 1999).

The possibility that endophyte toxins could influence animal production stimulated much research, especially with sheep in Canterbury (e.g., Fletcher 1986; Fletcher & Barrell 1984). Published endophyte research with dairy cattle commenced in 1991 in South Australia (Valentine *et al.* 1993). These workers rotationally grazed cows over one year on irrigated swards of pure Ellett perennial ryegrass, showing a small reduction (4%) in milk production in spring and a larger reduction in summer (14%) on endophyte-infected compared with endophyte-free pastures. New Zealand trial work with dairy cattle has been conducted at two DRC sites – the WestpacTrust Agricultural Research Station (WTARS) in Normanby, Taranaki (1992/93), and at Ruakura Agricultural Research Station near Hamilton (1993–1999).

This paper briefly reviews the results of the endophyte research with dairy cattle at the DRC sites. Detailed descriptions of the Taranaki (McCallum & Thomson 1994) and the Ruakura trials (Thom *et al.* 1994; Thom *et al.* 1997; Clark *et al.* 1999; Thom *et al.* 1999) have been published elsewhere.

## Trial methodology and design

### *WestpacTrust Taranaki Agricultural Research Station (WTARS) Trial*

Four, 1-ha paddocks (replicate blocks) were each divided into 0.25-ha strips and were sown with pure swards of Yatsyn 1, Embassy, Vedette and Grasslands Pacific perennial ryegrasses in different strips. Yatsyn 1, Embassy and Vedette were infected with wild-type endophyte (*Neotyphodium lolii*), while Grasslands Pacific was infected with a selected strain of endophyte (187BB). Treatment herds grazed the 4 strips of a particular ryegrass in a random sequence over 3 test periods, giving a 4 × 3 incomplete Latin Square design.

***Ruakura, Waikato Trials***

*Trial 1*: Four endophyte treatments were established in autumn 1993, using the same line of Yatsyn 1 perennial ryegrass:

(a) Endophyte-infected (85% of seeds contained the wild-type endophyte), established with white clover (H+C).
(b) Endophyte infected, established without white clover (H-C).
(c) Endophyte-free, established with white clover (L+C).
(d) Endophyte-free, established without white clover (L-C).

There were 10 paddocks (each 0.25 ha) of each treatment, plus another 3 ha of endophyte-free pastures for grazing before and after test periods. The trial spanned 3 years (April to March) and the clover treatments were eliminated for the final year. Pasture treatments were randomly arranged in paired paddocks within a 2 × 2 factorial design with five replicates.

*Trial 2*: Five ha of endophyte infected (95% of seeds contained the wild-type endophyte) Yatsyn 1 perennial ryegrass (H) and 5 ha of endophyte-free Yatsyn 1 were established (L) one year after the completion of Trial 1. Cows grazed on another 3 ha of endophyte-free ryegrass before and after each test period. The trial design was a 2 × 2 factorial with pasture containing low (L) and high (H) levels of endophyte-infected perennial ryegrass fed at two pasture allowances, nominally 25 and 45 kg DM/cow/day.

***Cows***

Cows were involved in short term trials in spring, summer and autumn, in Taranaki and Waikato (Table 1). The cows were grazed as one herd before each test period and milk production, liveweight and condition score data during this time were used as covariates. Cows were randomly allocated to treatment groups according to age, breed (where necessary), milksolids (MS) production from a recent herd test, liveweight, condition score, and calving date. In Trial 2 at Ruakura, the cows used in spring 1997 and autumn 1998 were all Jerseys; in spring 1998 each treatment herd contained eight Friesians and seven Jerseys and in summer 1999 the herds contained only Friesians.

***Pasture measurements***

(i) *WTARS*: Herbage mass was estimated before and after each grazing; pasture samples were cut to grazing height on 2 or 3 occasions during each test period for determination of botanical composition, lolitrem B and ergovaline (summer/autumn only) concentrations.
(ii) *Ruakura*: Trial 1: Herbage accumulation and pasture growth rates were estimated from exclosure cage measurements; pasture samples were collected at 1- to 2-month intervals to determine botanical composition, and herbage samples were collected monthly for determination of lolitrem B and ergovaline concentrations. Samples of ryegrass tillers were taken in spring, summer and autumn of each year to check for the presence of endophyte. All samples were cut to ground level.

**Table 1** Cow test periods, and details relating to cows used during DRC trials in Taranaki and the Waikato.

| Site | Cow test periods | Breed | Cows/treatment | Allowance (kg DM/cow/day) | Measurements |
|---|---|---|---|---|---|
| Normanby (Taranaki) | 8 days in spring, summer and autumn 1992/93 | Mainly Jersey x Friesians | 10 | Same across treatments | Milk volume<br>Milk composition (fat, protein and lactose)<br>Milksolids (fat + protein)<br>Cow liveweight<br>Cow condition score |
| Ruakura (Waikato) | Trial 1<br>21 days in spring, summer and autumn, in each of 3 years (1993–1996) | Mainly Jerseys | 15 | Same across treatments | As above, plus cow rectal temperatures and ryegrass staggers scores |
| | Trial 2<br>21 days in spring, summer or autumn, in each of 2 years (1997–1999) | Jerseys and Friesians | 15 | High (45) or low (25) | As for Trial 1, plus somatic cell counts (SCC) |

Trial 2: Herbage allowances were calculated from calibrated visual assessments of herbage mass made in each paddock before grazing. Ryegrass tillers were collected for endophyte screening before each test period, as were pasture samples for the determination of botanical composition; these samples were cut to ground level.

***Statistical analyses***

Analysis of variance and covariance models were used to test for treatment differences. Data obtained from individual cow measurements were considered as replicates for analysis of treatment differences. Somatic cell count data did not follow a normal distribution and were log transformed before analysis.

## Results

***WTARS***

*Pastures*: Available herbage mass in spring and autumn tended to be higher on Yatsyn 1 and Vedette pastures, so the grazing area was reduced and the stocking rates were adjusted to maintain similar grazing residuals on all treatments. Ryegrass contents were similar in all seasons averaging 77% of DM in spring and 93% in autumn.

Grasslands Pacific herbage consistently had the lowest concentrations of lolitrem B in all seasons (range 0.05–0.27 µg/g DM) compared with the other cultivars (range 0.21–1.21 µg/g DM). Average lolitrem B concentrations in spring (0.36 µg/g DM) were lower than in summer (0.88 µg/g DM) or autumn (March) (0.60 µg/g DM). Average lolitrem B concentrations were lowest in April at 0.19 µg/g DM. Ergovaline concentrations in Grasslands Pacific herbage were higher (range 0.88–1.50 µg/g DM) in summer and autumn (March) than in the other cultivars (range 0.25–0.70 µg/g DM), with differences less clear-cut in April (range over all cultivars 0.20–0.60 µg/g DM).

*Milk production*: Cultivar had no effect on milk volume, milk composition or milksolids production in spring, summer or autumn (March). In April a lower protein content in milk from cows grazing Yatsyn 1 contributed to a lower ($P<0.05$) milksolids production from this cultivar (0.48 vs 0.53 kg cow/day).

***Ruakura, Trial 1***

*Pastures*: Annual herbage accumulations showed no treatment differences, averaging 12.8, 13.9, and 13.8 t DM/ha in Years 1, 2 and 3, respectively. Ryegrass herbage yield was the dominant component of total yield showing few treatment effects. During an extended dry period in the summer/autumn of Year 1 (19 January to 19 April 1994), pasture growth rates were exceptionally low averaging 17 kg DM/ha/day. Ryegrass content was consistently highest (70–96% of DM) in winter/spring and lowest in late summer/early autumn (February/March) (25–60% of DM). The endophyte status of the pastures rarely affected their ryegrass content.

Endophyte infection levels in H treatments were usually >80% of plants. However, endophyte levels in L treatments increased with time, averaging 26% by the end of Year 1 (March 1994) and 50% of plants by the end of Year 3 (March 1996).

Spring (October) concentrations of lolitrem B in H treatments were <0.40 µg/g DM compared with zero in L treatments. Peak levels of lolitrem B occurred from February to April 1994, and February and December 1995 at 3.1, 2.4 and 1.2 µg/g DM, respectively. Comparable data for L treatments were 0.67, 0.62 and 0.39 µg/g DM. Ergovaline levels in spring averaged 0.38 µg/g DM in H compared with 0.15 µg/g DM in L treatments. Peak ergovaline concentrations occurred at the same time as those for lolitrem B, averaging 0.87, 0.65 and 1.60 µg/g DM in H treatments compared with 0.60, 0.35 and 0.65 in L treatments.

*Milk production*: Ryegrass endophyte had small effects on milk production, reaching statistical significance in 2 of the 9 test periods over 3 years. There were no significant effects of endophyte on milk production in Year 1 (1993/94), but in the second spring (1994) and the third summer (1995/96), milk production was reduced by endophyte (Table 2). In summer 1993/94 cows grazing L+C produced more milk than those on other treatments, causing a significant interaction between endophyte and clover treatments (Table 2). Similar effects occurred in summer 1994/95 and to a lesser extent in autumn 1994.

*Incidence of ryegrass staggers and heat stress*: Cows on the H treatments showed clinical symptoms of ryegrass staggers in autumn (March) 1994 and in summer 1994/95; however, in summer 1995/96, when milk production was reduced due to endophyte, no cows showed clinical symptoms of ryegrass staggers. No cows on L treatments showed clinical symptoms of ryegrass staggers. Heat stress was absent from all test periods and treatments did not change rectal temperatures of the cows.

***Ruakura, Trial 2***

*Pastures*: During the spring test periods the pastures were mainly ryegrass (73–85% of DM) and *Poa spp.* (2–11% of DM). In summer, however, ryegrass accounted for only 28–46% of DM; dead material (17–

34% of DM) and $C_4$ volunteer grasses including smooth witchgrass (*Panicum dichotomiflorum*) and summer grass (*Digitaria sanguinalis*) made up most of the remainder (8–33% of DM). In March 1998, there was 4 times as much $C_4$ volunteer grass in H (33% of DM) than in L (8% of DM); this trend was weaker in January 1999 (21 vs 13% of DM).

Endophyte infection in H pastures increased from 80% of the plants present in spring 1997 to 96% by summer 1999. Endophyte incidence in areas sown with endophyte-free ryegrass was usually less than 6% of plants, however, in March 1998 16% of plants were infected, compared with 95% in H pastures.

Only spring 1997 and summer 1998 herbage lolitrem B and ergovaline concentrations are available. Lolitrem B concentrations in spring H pastures averaged 0.19 µg/g DM but it was undetectable in L pastures. Lolitrem B concentrations were higher in summer 1998 at 1.72 and 0.62 µg/g DM for H and L pastures, respectively. Spring ergovaline concentrations in H pastures averaged 0.3 µg/g DM compared with zero in L pastures; summer 1998 concentrations of ergovaline were low and similar (0.1 µg/g DM) in H and L pastures.

*Milk production*: In spring 1997 (October) milk volume and milksolids (MS) yield were higher (P<0.05) for L than for H endophyte (Table 3). Cows on L endophyte gained more liveweight (P<0.05) and condition (P<0.05) than did those on H endophyte. As expected, cows on a high allowance produced higher milk volumes and MS, and higher liveweights and condition scores than those on low allowances; this trend was consistent throughout the trial (data not shown).

In autumn 1998 (March) milk volume was higher (P<0.001) from H compared with L endophyte, with a similar difference for MS yield (Table 3). Cows maintained liveweight and condition score over the three week trial period and there were no effects of endophyte.

In spring 1998 (October) there was no effect of endophyte on milk volume or MS yield (Table 3). Somatic cell counts (SCC) on all treatments were very low with an overall mean of 47 500 cells/ml. Cows on all treatments gained liveweight and condition score and there were no treatment effects on these variables.

In summer 1999 (January/February) there was no effect of endophyte on milk volume or MS yield (Table 3). SCC on all treatments were low averaging 78 600 cells/ml over the test period and there was no effect of endophyte level. Liveweight and condition score were similar for cows on all treatments.

*Incidence of ryegrass staggers and heat stress*: No clinical ryegrass staggers was noted during the test periods in spring 1997, spring 1998 or summer 1999.

**Table 2** Effect of high (H) and low (L) endophyte pastures on milk volume (litres/cow/day) (MV) and milksolids (kg/cow/day) (MS) production in spring and summer during Trial 1. Means for spring represent main effects; means for summer 1993/94 and 1994/95 represent interaction effects. The clover (± C) treatment was removed in July 1995; means for summer 1995/96 represent main effects.

| | | ------------- Treatment ------------- | | | | |
|---|---|---|---|---|---|---|
| | | H – C | H + C | L – C | L + C | SED |
| **Spring** | | | | | | |
| MV | 1993 | 16.9 | - | 16.4 | - | 0.41 NS |
| MS | | 1.54 | - | 1.53 | - | 0.05 NS |
| MV | 1994 | 16.3 | - | 17.3 | - | 0.38 ** |
| MS | | 1.58 | - | 1.65 | - | 0.04 NS |
| MV | 1995 | 16.7 | - | 16.9 | - | 0.22 NS |
| MS | | 1.45 | - | 1.45 | - | 0.03 NS |
| **Summer** | | | | | | |
| MV | 1993/94 | 9.2 | 9.2 | 8.7 | 10.30 | 0.29 * |
| MS | | 0.90 | 0.93 | 0.87 | 1.02 | 0.03 * |
| MV | 1994/95 | 7.7 | 7.6 | 8.2 | 9.4 | 0.24 *** |
| MS | | 0.75 | 0.75 | 0.80 | 0.93 | 0.03 ** |
| MV | 1995/96 | 8.4 | - | 8.9 | - | 0.22 * |
| MS | | 0.87 | - | 0.91 | - | 0.03 NS |

Significance levels: NS = not significant, * P<0.05, ** P<0.01, *** P<0.001.

**Table 3** Effect of high (H) and low (L) endophyte pastures on seasonal milk volume (litres/cow/day) (MV) and milksolids (kg/cow/day) (MS) production (Trial 2).

| | | ----- Treatment ----- | | |
|---|---|---|---|---|
| | | H | L | SED |
| **Spring 1997** | | | | |
| | MV | 14.4 | 15.1 | 0.28 * |
| | MS | 1.47 | 1.53 | 0.03 * |
| **Autumn 1998** | | | | |
| | MV | 8.5 | 7.4 | 0.17 *** |
| | MS | 0.89 | 0.81 | 0.02 ** |
| **Spring 1998** | | | | |
| | MV | 18.8 | 18.9 | 0.35 NS |
| | MS | 1.57 | 1.56 | 0.04 NS |
| **Summer 1999** | | | | |
| | MV | 11.1 | 11.0 | 0.23 NS |
| | MS | 0.89 | 0.88 | 0.02 NS |

Significance levels: NS = not significant, * = P<0.05, ** = P<0.01, *** = P<0.001

During summer 1998, however, several cows on the H endophyte showed intermittent ryegrass staggers but of low severity, and no staggers were recorded on L pastures.

Mean cow rectal temperatures were 38.5, 38.6, 38.4 and 39.4°C during the spring 1997, summer 1998, spring 1998 and summer 1999 test periods, respectively, and there were no effects of endophyte level.

## Discussion

A total of 16 short-term tests of the effects of endophyte on milk production have been run at DRC sites over 7 years; three tests were conducted in Taranaki and 13 at Ruakura. This work has not shown consistent, strong effects of endophyte on milk production.

Exposing cows to different ryegrass cultivars and endophyte alkaloid combinations during the spring, summer and autumn of one lactation in Taranaki, did not affect milk volume or milksolids production. There was a wide range of lolitrem B concentrations in the herbage eaten by the cows but clinical ryegrass staggers was not observed as lolitrem B concentrations were low to moderate and below 2 µg/g DM, the level recognised by Prestidge & Gallagher (1989) as likely to cause ryegrass staggers. Ergovaline concentrations were also generally low to moderate during the test periods, except in Grasslands Pacific herbage in February (1.50 µg/g DM), when concentrations of 1.0 µg/g DM are thought necessary to induce heat stress symptoms in cattle (Easton *et al.* 1996). Such concentrations also need to be present during periods of high ambient temperature (>30°C) and humidity to precipitate clinical symptoms of heat stress in cattle. These conditions were not present at least for the ambient temperature range reported (McCallum & Thomson 1994) of -2 to 26°C.

In the Ruakura trials, negative effects (5–6%) of endophyte on milk production were recorded in spring 1994 and spring 1997, but there were no effects in spring 1993, spring 1995 or spring 1998 (Tables 2, 3). Negative effects in summer/autumn were confined to Year 3 of Trial 1, and in autumn 1998 (Trial 2), cows on H pastures unexpectedly produced more milk than those on L pastures. These effects of endophyte could not be strongly linked to herbage concentrations of lolitrem B or the development of clinical symptoms of ryegrass staggers. For example, lolitrem B concentrations were low (<0.40 µg/g DM) in spring 1994 compared with the following summer (3.1 µg/g DM); clinical ryegrass staggers were not recorded in spring 1994 but were observed in some cows on the H pastures in the following summer. Although endophyte reduced milk volume in summer 1995/96 (Table 2), cows did not show clinical ryegrass staggers, possibly because peak herbage lolitrem B concentrations at this time were relatively low (1.2 µg/g DM) compared with the critical level identified by Prestidge & Gallagher (1989). Valentine *et al.* (1993), working in South Australia with irrigated pure swards of the New Zealand cultivar Ellett also found a similar spring reduction (4%) in milk volume due to endophyte, and when herbage lolitrem B concentrations were low (averaging 0.23 µg/g DM). In the following summer, however, Valentine and co-workers reported a greater reduction in milk volume (14%) due to endophyte than was found in the present trial (5% in summer 1995/96) as the only warm season response, following the removal of white clover.

No clinical heat stress symptoms relating to endophyte treatment were observed in any test periods during the Ruakura trials, which was consistent with generally low herbage ergovaline concentrations (<1.0 µg/g DM), and with the lack of effect of endophyte treatment on cow rectal temperatures.

The intensity of the summer peaks in herbage lolitrem B concentrations in Trial 1 (Ruakura) seemed to correlate with summer rainfall, being highest in the driest summer (Year 1, 25% below average rainfall) and lowest in the wettest summer (Year 3, 35% above average rainfall).

Another feature of Trial 1 was the rapid and increasing contamination of the endophyte-free areas with volunteer endophyte-infected ryegrass, however, removal of seedheads and double spraying of the treatment areas before drilling kept contamination in L areas of Trial 2 to a minimum (usually <6%). The contamination of endophyte-free areas in Trial 1 could have modified milk responses to endophyte, although at least for lolitrem B, peak levels in herbage remained in a narrow range (0.45 to 0.67 µg/g DM) in L treatments compared with H (1.2 to 3.1 µg/g DM). Sources of contamination have been identified in further work (van Vught & Thom 1997) and include reseeding from existing endophyte-infected ryegrass before renewal, transport of endophyte-infected seed in cow dung, and survival of existing endophyte- infected ryegrass which was usually partially covered by dung when sprayed with herbicide.

## Conclusions

- Milk production reductions due to endophyte were small (<6%) and inconsistent over 7 years of testing.
- Contamination of endophyte-free areas in Trial 1 in Waikato reached 50% after 3 years, but milk production trends were similar to those for Trial 2 at the same site, when contamination was low (6–16%).
- Two of the three negative milk production responses to endophyte occurred in spring when levels of lolitrem B and ergovaline were low. Further research is required to identify the reasons for these effects.
- Ryegrass staggers occurred at Ruakura during three of the five warm season (summer/autumn) tests during Trials 1 and 2, but staggers and negative responses to endophyte did not occur together.
- Heat stress, as indicated by cow rectal temperatures, was absent during all test periods.
- Research into the effects of endophyte on milk production is limited by the low number of New Zealand testing sites where tests have been made. Although long-term farmlet trials might be desirable

they require much more resources than short-term trials. Farmlet testing will also face the problem of how to keep low endophyte areas productive if insect damage occurs.

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