Ryegrass staggers and liveweight gain of ewe lambs and hoggets grazing four combinations of perennial ryegrass and strains of endophyte

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Abstract

Ryegrass staggers and live weight gain of Coopworth ewe lambs or hoggets were assessed over four eight week trials (two summer, two autumn) for four combinations of perennial ryegrass and strains of endophyte: Arrow with standard (SE, wild type) endophyte, Trojan with NEA3 endophyte, Trojan with a mixture of NEA2 and NEA3 endophytes, and Rohan with a mixture of NEA2 and NEA6 endophytes. In each trial, three replicates of 10 to 12 sheep were assigned to 0.3 ha plots of each cultivar x endophyte strain combination, with herbage mass reduced from 3-4000 kg DM/ha to 1500 kg DM during the eight weeks. Severe ryegrass staggers occurred in all four trials in Arrow SE, with a mean ryegrass staggers score ranging between 1.65 to 2.8 (0 to 5 scale). Minor ryegrass staggers occurred in Trojan NEA3, Trojan NEA2/3, Rohan NEA2/6 in two of the four trials, but only with a low mean score (<0.4). Live weight gain was lower in Arrow SE than the other cultivars in three of the four trials. Trojan NEA3 had live weight gain that was different from Trojan NEA2/3 (+27 g/head/day higher in Trojan NEA2/3) on one trial only. The perennial ryegrass cultivar x novel endophyte strain combinations Trojan NEA3, Trojan NEA2/3 and Rohan NEA2/6 showed liveweight gain that was similar or greater than the standard endophyte control, and caused minimal ryegrass staggers in 'worst-case scenario' conditions designed to induce this.

Keywords: endophyte; ryegrass staggers; liveweight gain; perennial ryegrass

Introduction

The endophyte *Neotyphodium lolii* may form a symbiotic relationship with perennial ryegrass (*Lolium perenne*), the most commonly sown grass in New Zealand (di Menna et al. 2012). The production of a range of alkaloids, including lolitrem B, peramine, ergovaline and epoxy-janthitrems, in the plant through this relationship, offer advantages for pasture production and persistence, through protection against insect attack and overgrazing (Edwards et al. 1993; Popay & Hume 2011). However, there may also be negative effects of alkaloids on livestock performance. A high intake of lolitrem B is associated with ryegrass staggers, while ergovaline intake is linked to heat stress (Fletcher & Easton 2007; Nicol & Klutz 2015). Recently strains of *Neotyphodium lolii* have been discovered where ergovaline rather than lolitrem B is the dominant alkaloid, and perennial ryegrass cultivars containing these strain of endophytes are commercially available (di Menna et al. 2012). An important part of assessing the benefits of these endophytes to livestock production systems is to assess animal health and livestock performance. The objective of this study was to compare ryegrass staggers and liveweight gain of four combinations of perennial ryegrass and strains of endophyte , that produce different alkaloid profiles of lolitrem B and ergovaline.

Materials and methods

Experimental site and treatments

Three replicated 0.3 ha plots of four combinations of perennial ryegrass and strains of endophyte (n = 12 plots in total) were established at an irrigated site on Lincoln University's farm, Ashley Dene, located near Burnham, Canterbury (-43.65 °N 172.33 °E) in April 2012. The soil type was a stony, free-draining Balmoral soil with low-water-holding capacity. The four combinations of perennial ryegrass and strains of endophyte were: (1) Arrow with standard (wild type) endophyte (Arrow SE); (2) Trojan with NEA3 endophyte; (Trojan NEA3); (3) Trojan with a mixture of NEA2 and NEA3 endophytes (Trojan NEA2/3); and (4) Rohan with a mixture of NEA2 and NEA6 endophytes (Rohan NEA2/6) which is what is marketed in New Zealand under the "Rohan NEA2" brand. For establishment, plots were ploughed, cultivated and perennial ryegrass seed was sown at 20 kg/ha, with no white clover, using a roller drill on 15 March 2012. The plots were grazed using best practice up until one month prior to the trials beginning. Plots were irrigated and fertilised with 50 kg N/ha as urea one month prior to the start of each trial, so that the starting herbage mass was 2500 to 4000 kg DM/ha, as close to the target range of 3000-3500 kg DM/ha as possible. Each plot was grazed with 12 Coopworth ewe lambs or hoggets over four eight week trials: Summer 2012 (22 November 2012 to 17 January 2013, lamb live weight = 33.7 kg); Autumn 2013 (2 February to 19 April 2013, lamb live weight = kg 35.4); Summer 2013 (19 November 2013 to 13 February 2014, hogget live weight = 55.9 kg); and Autumn 2013 (11 March to 5 May 2014, lamb live weight = 32.9 kg). Plots were not irrigated during each trial. This management was designed to create conditions conducive to ryegrass staggers and it is recognised that it is not appropriate for maximising live weight gain.

Animal measurements

All sheep were weighed after a 12-hour fast at the start, middle (week 4) and end (week 8) of each trial. All sheep were scored every two weeks for ryegrass staggers on a 0-5 ascending scale (Keogh 1973). Individual animals were removed from the trial to safe pasture when they reached score 3 to 4 on two consecutive observations. These animals were replaced with non-trial animals to maintain grazing pressure.

Herbage measurements

Herbage mass on offer to animals was assessed every two weeks by taking 50 compressed pasture heights with a calibrated rising plate meter (RPM) (JenQuip, Filips EC-09, Electronic Folding Plate Meter). The composition of the herbage on offer was assessed by herbage samples cut to ground level every two weeks. It is noted that sampling to ground level may not accurately reflect the diet of the animals, as metabolisable energy and endophyte alkaloid concentrations vary through the ryegrass plant, and are lower and higher respectively in the basal stems of grass plants, compared to the leafy tips of grass plants (Nicol & Klotz 2015). The herbage samples were frozen at -20°C, and later freeze-dried for chemical analysis. Herbage samples were ground through a 1-mm sieve (ZM200, Retsch). One sub-sample was analysed for Digestible Organic Matter Digestibility DOMD, using near-infrared spectroscopy (Corson et al. 1999) and metabolisable energy determined from the equation: MJME/kg DM = DOMD (g/kg DM) x 0.016 (McDonald et al. 2002). A second sub-sample from all trials was analysed using high-performance liquid chromatography (HPLC) for ergovaline, lolitrem B and janthitrem using a 150 mm x 2.1 mm Themo Hypersil Gold 1.9 µm HPLC column fitted to an Agilent series 1200 high-performance liquid chromatograph (Agilent, Walbronn). This analysis gave the relative rather than absolute concentration of each alkaloid, expressed as relative ion intensity. In addition, herbage samples collected in the second and fourth week of the Autumn 2013 trial were analysed for ergovaline and lolitrem B concentration (ppm) using methods described by Rasmussen et al. (2007).

Statistical analysis

Herbage and animal data for each trial were analysed by one-way ANOVA using Genstat 15 (VSN International Ltd., Hemel Hempstead, UK). In each case, plot was used as replicate and the data analysed were the means of each group of animals or herbage samples for a plot.

Results

Herbage

Mean herbage mass ranged from 1878 to 3754 kg DM/ha across trials, but was unaffected by cultivar in any trial (Table 1). Metabolisable energy content of herbage cut to ground level ranged from 9.5 to 11 MJME/kg DM but was unaffected by cultivar in any trial (Table 1).

Relative ion concentrations of ergovaline and lolitrem B of the four cultivars are shown in Table 1. Ergovaline was present in all cultivars in all four trials. In Summer 2012, ergovaline concentration was greater in Arrow SE, Trojan NEA3, Rohan NEA2/6 than Trojan NEA2/3 (Table 1). In Summer 2013, ergovaline concentration was higher in Trojan NEA3 than Rohan NEA2/6 and Arrow SE, which were higher than Trojan NEA2/3. In Autumn 2014, ergovaline concentration was higher in Trojan NEA3

Table 1 Mean herbage mass (kg DM/ha), metabolisable energy (MJ ME/kg DM) and ergovaline and lolitrem B concentration (relative ion intensity x 10^6) of four combinations of perennial ryegrass and strains of endophyte in four separate trials. Data are averaged over samples collected over entire eight weeks of each trial. LSD = least significant difference (α = 0.05). Means followed by different letters within the same row are significantly different according a LSD test (α< 0.05) following a significant ANOVA.

	Arrow SE	Trojan NEA3	Trojan NEA2/3	Rohan NEA2/6	P value	LSD
Herbage mass						
Summer 12	3754	3644	3493	3416	0.73	789
Autumn 13	2603	2354	2346	1836	0.21	786
Summer 13	2396	2175	2147	1878	0.07	621
Autumn 14	2905	2703	2288	2637	0.09	487
ME						
Summer 12	10.1	10.5	10.3	10.6	0.34	0.64
Autumn 13	10.1	10.5	10.2	10.5	0.44	0.50
Summer 13	9.7	9.8	9.6	9.7	0.56	0.53
Autumn 14	10.9	11.1	10.9	11.2	0.45	0.46
Ergovaline						
Summer 12	3.80a	4.47a	2.43b	3.33ab	0.03	1.25
Autumn 13	1.33	2.34	1.22	1.98	0.32	1.54
Summer 13	2.38b	3.61a	2.05b	1.11c	<0.01	0.71
Autumn 14	1.53b	3.74a	2.19b	1.74b	<0.01	1.07
Lolitrem B						
Summer 12	0.94a	0b	0.01b	0b	<0.01	0.088
Autumn 13	1.09a	0b	0.04b	0.01b	<0.01	0.081
Summer 13	1.12a	0b	0.02b	0b	<0.01	0.208
Autumn 14	1.15a	0b	0b	0.01b	<0.01	0.165

Table 2 Mean liveweight gain (g/head/d) and ryegrass staggers score (0-5 scale) over eight weeks for four combinations of perennial ryegrass and strains of endophyte in four separate trials. LSD = least significant difference (α = 0.05). Means followed by different letters within the same row are significantly different according a LSD test (α< 0.05) following a significant ANOVA.

	Arrow SE	Trojan NEA3	Trojan NEA2/3	Rohan NEA2/6	P value	LSD
Liveweight gain						
Summer 12	28b	49b	76a	75a	<0.01	21.1
Autumn 13	82b	142a	157a	123a	<0.01	43.6
Summer 13	35b	45b	46b	130a	0.04	50.3
Autumn 14	130	161	134	150	0.16	52.8
Ryegrass staggers						
Summer 12	1.63a	0.07b	0.22b	0.12b	<0.01	0.15
Autumn 13	3.27a	0.01a	0.31b	0.16b	<0.01	0.39
Summer 13	1.50a	0b	0b	0b	<0.01	0.14
Autumn 14	1.60a	0b	0b	0b	<0.01	0.13

than all other cultivars. Lolitrem B was always present in Arrow SE, and present in lower but inconsistent levels in Trojan NEA2/3 and Rohan NEA2/6 (Table 1). Lolitrem B was detected in samples of Rohan NEA2/6 in Autumn 2012 and Autumn 2013, and in Trojan NEA2/3 in Summer 2012, Autumn 2013 and Summer 2013, but always in significantly lower concentrations than in Arrow SE (Table 1). Lolitrem B was never detected in Trojan NEA3 and no janthitrem was detected in any of the herbage samples. For samples collected from week two and six of the Autumn 2013 trial, where the actual concentration was measured, the concentration of ergovaline was higher (P<0.01) in Trojan NEA3 (1.46 mg/kg DM) than Arrow SE (0.80 mg/kg DM), Trojan NEA2/3 (0.72 mg/kg DM) and Rohan NEA2/6 (0.85 mg/kg DM). For the same samples, the concentration of lolitrem B was higher (P<0.01) in Arrow SE (2.22 mg/kg DM) than Trojan NEA2/3 (0.33 mg/kg DM) and Rohan NEA2/6 (0.30 mg/kg DM), while no lolitrem B was detected in Trojan NEA3.

Animals

In Summer 2012, liveweight gain was higher in sheep grazing Rohan NEA2/6 and Trojan NEA2/3 than Arrow SE and Trojan NEA3 (Table 2). In Autumn 2013, liveweight gain was lower in sheep grazing Arrow SE than all other cultivars (Table 2). In Summer 2013, liveweight gain was higher in Rohan NEA2/6 than all other cultivars (Table 2). Ryegrass staggers occurred in all trials in sheep grazing Arrow SE, with a mean ryegrass staggers score (0 to 5 scale) ranging from 1.50 to 3.27 (Table 2). Ryegrass staggers was observed in sheep grazing Trojan NEA3, Trojan NEA2/3 and Rohan NEA2/6 in Summer 2012 and Autumn 2013, but then only with a low score (<0.31). No ryegrass staggers was observed in sheep grazing these three cultivars in Summer 2013 and Autumn 2014 trials.

Discussion

Ryegrass staggers occurred in all trials in sheep grazing Arrow SE with severe incidence in Autumn 2013. This result confirms the strong relationship between intake of the tremorgen lolitrem B and ryegrass staggers (Fletcher & Easton 2007). In contrast, negligible ryegrass staggers was observed in sheep grazing Trojan NEA3, Trojan NEA2/3 and Rohan NEA2/6. In the first two trials (Summer 2012 and Autumn 2013), when severe staggers were seen in Arrow SE, some tremors were observed in Trojan NEA2, Trojan NEA2/3 and Rohan NEA2/6. Here, it was observed in a small number of animals (<10 of 36 in each trial) and with a very low mean ryegrass staggers score (<0.31 across the eight weeks of trial). However, no ryegrass staggers were detected in Trojan NEA3, Trojan NEA2/3 or Rohan NEA2/6 in the last two trials (Summer 2013 and Autumn 2014). The reason for low incidence of ryegrass staggers detected in these three cultivars is unclear. The tremorgen lolitrem B was detected on occasions in herbage sampled from Trojan NEA2/3 and Rohan NEA2/6 pasture but always in very low concentration relative to Arrow SE (Table 1). Ryegrass staggers has been detected occasionally in lambs grazing perennial ryegrass infected with the AR37 endophyte strain (Fletcher 1999; Fletcher & Sutherland 2009). However, this has been associated with the production of janthitrems (Tapper & Lane 2004; Fletcher & Sutherland 2009), and no janthitrems were detected in the herbage sampled from this trial. Tremors consistent with minor ryegrass staggers have also been detected in lambs grazing perennial ryegrass infected with the non-toxic endophyte AR1, and assigned to tremorgens other than lolitrem B (Fletcher 1999).

Liveweight gain of ewe lambs and hoggets was variable and ranged from 28 to 157 g/head/day (Table 2). These liveweight gain values are within the range detected (-87 to 366 g/head/day) for young sheep grazing tall fescue and perennial ryegrass swards in nine trials conducted in the Hawkes Bay and Canterbury (Hyslop et al. 2000). There was a consistent trend of the lowest liveweight gain occurring in Arrow SE (Table 2). The lower liveweight gain probably reflects the lower DM intake associated with high incidence and severity of ryegrass staggers in Arrow SE (Nicol & Klotz 2015). Consumption of ergovaline may also contribute to lower liveweight gain (Layton et al. 2004), however, the concentration of ergovaline was similar to, or in two cases lower, in Arrow SE than other cultivars in each trial (Table 1).

Detecting the effect of a specific endophyte or cultivar or endophyte on liveweight gain is difficult from this study as changes in endophyte strain were generally confounded with changes in cultivar. However, some conclusion about the effects of a specific endophyte on livestock performance can be obtained from a comparison of Trojan containing NEA3 alone or a combination of NEA2 and NEA3. Inclusion of NEA2 in the mixture with NEA3 in the same cultivar resulted in some lolitrem B production and a lower ergovaline concentration but did not affect herbage mass or ME (Table 1). Despite ryegrass staggers occurring at low level in two trials in which NEA2 was included, there were only small differences in liveweight gain between Trojan NEA3 and Trojan NEA2/3, with liveweight gain different (+27 g/head/day higher in Trojan NEA2/3) in Summer 2013 only (Table 2). This suggests that including NEA2 in a mixture with NEA3 will achieve similar levels of livestock performance in the Trojan cultivar.

These liveweight gain data are from pure swards of perennial ryegrass, grown to a high herbage mass and then stocked for a relatively long period. Higher liveweight gain would have been expected if white clover was part of the pasture mixture (Hyslop et al. 2000; Nicol & Edwards 2011). It has been shown that high liveweight gain is typically achieved where pastures are kept relatively short with a high proportion of leaf in the sward, which in turn can improve clover content; however, the objective here was to determine the effect of the endophyte-cultivar combination *per se*, so pastures were sown as pure swards of ryegrass with a relatively low leaf:stem ratio and ME, and stocked for eight week-periods to maximise any effects on animal health.

Conclusion

The combinations of perennial ryegrass and strains of novel endophyte Trojan NEA3, Trojan NEA2/3 and Rohan NEA26 showed liveweight gain that was similar or greater than Arrow SE, the standard endophyte control. Lambs grazing Trojan NEA3, Trojan NEA2/3 and Rohan 2/6 showed minimal ryegrass staggers in conditions specifically designed to induce this.

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