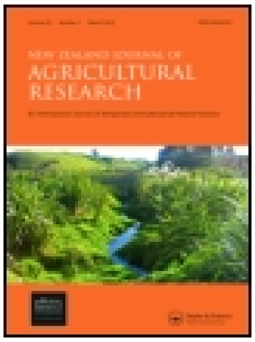

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RESEARCH ARTICLE

Comparing response of ryegrass-white clover pasture to gibberellic acid and nitrogen fertiliser applied in late winter and spring

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ABSTRACT
The objective of this study was to compare the effect of one or two applications of gibberellic acid (GA) or nitrogen (N) fertiliser on dry matter (DM) yield and composition of perennial ryegrass-white clover pasture. Pasture was treated with 0 or 8 g a.i./ha of GA and 0 or 50 kg/ha N fertiliser in a 2 × 2 factorial design in late winter and spring. One application of N fertiliser increased DM yield by increasing leaf mass and tiller density of perennial ryegrass while one application of GA increased DM yield by increasing perennial ryegrass and white clover yield. There was no DM yield response to two applications of GA, though two applications of N did increase DM yield. A single GA application in late winter increased fibre and reduced protein concentration in ryegrass. The use of GA to increase DM yield with reduced herbage protein concentration may have reduced environmental impact through reducing N intake of livestock.

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Introduction

Maintaining an adequate feed supply for pastoral-based livestock systems requires careful management and an understanding of plant responses to changing environmental conditions. In many temperate regions throughout Europe, South America and Australasia, binary mixtures of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) are sown for livestock grazing due to their high pasture yield, ability to withstand a wide range of grazing conditions and high feeding value (Kemp et al. 1999). However, at critical times for livestock feeding, such as following calving or lambing in late winter and early spring, pasture growth rates can be insufficient to meet livestock requirements.

Despite low temperatures at this time, it has been shown that the application of exogenous gibberellic acid (GA) can stimulate grass growth (Morgan & Mees 1958; Finn & Nielsen 1959; Zaman et al. 2014) and increase feed supply, as can application of nitrogen (N) fertiliser (Harris et al. 1996; Parsons et al. 2013). However, the associated increase in herbage N content with N fertiliser may come at an environmental cost if N fertiliser results in increased N intake. High N intake of intensively grazed animals leads to increased excretion of N in the urine (Higgs et al. 2012) and increased risk of nitrate leaching under urine

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patches (Li et al. 2012). Recently, use of exogenous GA has been investigated as a means of reducing environmental N losses (Whitehead & Edwards 2015) as it has been associated with reduction in herbage N content in a number of studies (Morgan & Mees 1958; Finn & Nielsen 1959; Brown et al. 1963; McGrath & Murphy 1976; Zaman et al. 2014), and has potential to reduce N intake and lower urinary N excretion.

In spite of evidence for improved herbage growth following GA application, adoption by farmers has been limited. This is thought to be due to previous high cost of the product at high rates of GA (63–600 g a.i./ha), and the risk of longer term reductions in DM yield (Matthew et al. 2009). However, manufacture of GA has become more economical and rates required to elicit a response are lower (5–10 g a.i./ha) than those previously studied (Matthew et al. 2009). Indeed, it was recognised that the high rates of GA may have been responsible for observed yield reductions. A study using low GA rates (8 g a.i./ha) showed increased pasture height and improved DM yields above mowing height in spring (Matthew et al. 2009). Although in the study of Matthew et al. (2009) residual effects of GA on subsequent regrowth were not statistically significant, the authors observed a tendency for lower DM yields and reduced tiller numbers in plots where GA was used without N fertiliser. The evidence that an interaction between GA and N existed led the authors to highlight the need for future consideration of these interactions and, in particular, how N may reduce negative effects of GA on subsequent regrowth.

The purpose of this study was to compare pasture growth response to GA, N fertiliser and their interaction following one or two applications in late winter and early spring. Effects on botanical composition, grass morphology and nutrient composition were included to quantify treatment effects on DM yield parameters.

Materials and methods

Experimental site and pastures

The experiment was conducted at Iversen Field, Lincoln University, Canterbury, New Zealand, on a Wakanui silt loam soil (43°38′S. 172°27′E; 10 m above sea level) between August and November 2009. The experimental area was situated within a 0.9 ha pasture that had been sown, following cultivation, with a mixture of an early maturing perennial ryegrass (cv. Meridian, heading date –17 days, AR1 endophyte) and white clover (cv. Denmark) at 15 and 3 kg/ha, respectively, on 7 November 2008. Soil tests prior to commencement of the trial showed: pH = 5.6; Olsen P = 13 mg/L; and sulfate sulfur = 6 mg/kg. Pastures had previously been managed by rotational grazing with sheep to maintain herbage mass in the range 1200 to 1500 kg DM/ha.

Experimental design

The experiment consisted of a 2 × 2 factorial design with two regrowth cycles laid out in four randomised blocks each 8 × 8 m. The two treatments used were: 1, N fertiliser at two rates of 0 or 50 kg N/ha as urea (46% N); and 2, GA at rates of 0 or 8 g a.i./ha as ProGibb-SG containing 40% GA_3 (Nufarm Ltd). Each block was divided into four 2 × 8 m plots which were randomly allocated one of the four treatments. All plots were mown with a rotary lawnmower to a uniform height of 4.5 cm 24 hours prior to treatment application.

ProGibb-SG and non-ionic surfactant Contact Xcel (Nufarm Ltd) were mixed with water at rates of 20 g and 25 ml per hectare, respectively, and applied to experimental areas with a knapsack sprayer on 5 August 2009. Urea was applied by hand-spreading within 48 hours of mowing. Measurements were carried out between 33 and 41 days after GA and N treatment application.

On completion of measurements following one application, the experimental site was set-stocked with sheep from 18 September to 2 October 2009. On 3 October 2009, sheep were removed and plots were mown with a rotary lawnmower to a uniform height of 4.5 cm and the GA and N treatment applications were repeated on 5 October 2009. A similar regrowth interval was used following the second application of GA and N treatments. Daily climate data were sourced from the NIWA database for the Lincoln Broadfields site (Station H32645) located 1 km from the experimental area.

Herbage measurements

Dry matter yield

Accumulated DM yield to ground level and above mowing height (4.5 cm) was determined at the end of each regrowth period. Dry matter yield to ground level was determined on 7 September and 11 November 2009 by harvesting two 0.2 m^2 quadrats per plot to < 1 cm stubble height using hand shears. After harvesting, each sample was thoroughly mixed, and soil and faecal matter removed. A subsample of 50 g fresh weight was removed to determine botanical composition. The remaining bulk sample was oven dried at 65 °C until it reached a constant weight. The sum of the subsample and bulk sample was used to calculate DM yield.

Dry matter yield above mowing height was determined at the end of each regrowth by mowing (4.5 cm height) a 3.2 m^2 strip within each plot. Total fresh matter (FM) of the mown herbage was recorded and a subsample of approximately 50 g of fresh herbage was removed, weighed and dried at 65 °C to a constant weight for DM% determination. Compressed pasture height was determined from 20 readings per plot using a rising plate meter (Jenquip F150 Electronic Pasture Meter) prior to mowing.

Sward characteristics

The subsamples from quadrat cuts were manually separated into vegetative and flowering ryegrass tillers, white clover, weed and dead material. The number of vegetative and flowering tillers were recorded. All components were then oven dried at 65 °C until they reached a constant weight and dry weights recorded. Botanical composition on DM basis was then determined. Mean tiller weight of vegetative and flowering ryegrass was calculated as the dry weight of each category divided by the number of tillers in the sample. Tiller density was calculated by multiplying average tiller weight (g DM/tiller) by the content of ryegrass in the pasture mass.

Tiller morphology

Individual plants were sampled by extracting 10 whole plants from each plot by cutting below ground level using a knife. From each plant, two primary tillers with intact crowns were carefully separated. Each tiller was dissected into leaf, pseudostem and truestem (crown and internodes), and length recorded. For each tiller, the length of the youngest fully emerged leaf from ligule to leaf tip and the width at the widest point were measured with a ruler. The leaf, pseudostem and truestem components of tillers were then oven dried at 65 °C, weighed and mass per tiller of each component determined.

Nutritive and mineral composition

Leaf and sheath material above 4.5 cm was sampled at 15:30 hr. Herbage clipped with hand shears was immediately placed on ice and transferred to a –20 °C freezer within 30 minutes of sampling. Frozen herbage was freeze dried and any clover and weed material was carefully removed from the dried sample before grinding through a 1 mm sieve (Retsch ZM200 rotor mill, Retsch GmbH). Ground perennial ryegrass was scanned for crude protein (CP), soluble sugars and starch (SSS), crude fibre (NDF), acid detergent fibre (ADF) organic matter (OM) and in vitro digestibility of organic matter in the DM (DOMD) using near infrared spectrophotometry (NIRS, Model: FOSS NIRSystems 5000) as described by Bryant et al. (2012). Nitrogen content was calculated by dividing CP by 6.25.

Separate herbage samples were collected for mineral analysis by harvesting a 30 × 30 cm quadrat of pasture to ground level on 15 September. Clover and weeds were removed from perennial ryegrass and discarded. The ryegrass samples were washed thoroughly with deionised water and placed in a drying cabinet at 105 °C until a constant weight was obtained. Herbage was dissolved using microwave digestion in 8 mL of Aristar nitric acid (± 69%), filtered using Whatman 52 filter paper (pore size 7 μm), and diluted with milliQ water to a volume of 25 mL. Concentrations of boron (B), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphours (P) and zinc (Zn) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES Varian 720 ES). Wageningen reference soil (ISE 921) and plant (IPE 100) material were analysed for quality assurance (Houba et al. 1998). Recoverable concentrations were 91%–108% of the certified values. The molar equivalents of Ca, Mg and K were used to calculate the ratio of K/(Mg + Ca).

Statistical analysis

For each regrowth, differences in DM yield, nutrient composition and botanical composition were analysed for variance using Genstat (v. 12.2 VSN international) with GA and N fertiliser and their interaction as fixed terms and blocks as the random term. Where interactions were significant, means were separated using Fisher's protected LSD test.

Results

Climate conditions for the experiment are presented in Table 1. Rainfall was greater following the second application than after the single application. Day length increased from 9.50 to 11.50 hr/day during the first regrowth and from 12.40 to 14.46 hr/day during the second regrowth period. Growing degree days (GDD) between mowing date and harvest

Table 1 Climatic conditions during the two regrowth periods from 5 August to 18 September and 3 October to 18 November 2009.

	Regrowth period	
	5 August to 18 September	3 October to 18 November
Rainfall (mm)	51	80
Mean max temperature (°C)	15.2	15.4
Mean min temperature (°C)	4.3	4.5
Mean 10 cm soil temperature (°C)	8.4	12.3
Range in 10 cm soil temperature (°C)	4.7–12.4	7.1–17.6

date for the first and second application of GA or N treatments were 175 °C and 237 °C, respectively (assuming a base temperature of 4 °C; Hutchinson et al. 2000).

Dry matter yield

Dry matter yield to ground level in the first regrowth cycle was increased by 407 kg DM/ha and by 217 kg DM/ha after one application of GA or N respectively ($P < 0.05$, Table 2). At rates of 8 g a.i. GA and 50 kg N/ha this equated to DM responses of 50.8 kg DM/g GA and 4.3 kg DM/kg N. Above mowing height the DM response to GA was still apparent, increasing DM yield by 360 kg DM/ha ($P < 0.05$). However, the response to N above 4.5 cm (138 kg DM/ha) was no longer significant. Dry matter yield for the second regrowth was not affected by GA at either mowing height or ground level. By contrast, DM yield was increased ($P < 0.05$) by two applications of N regardless of whether measurements were to ground level or mowing height, giving respective advantages of 1119 and 756 kg DM/ha over plots without N. Response to GA or N above ground level in the second regrowth cycle after two applications were −5.9 kg DM/g GA and 22.4 kg DM/kg N, respectively. There was an interaction between N and GA above mowing height for DM yield in the second regrowth, indicating that response to N was reduced when applied with GA.

Sward characteristics

Within a week of the first GA application, treated plots could be visibly differentiated from untreated plots. Treated plots visually appeared more yellow and taller than plots without

Table 2 Dry matter yield (kg DM/ha) to ground level (< 1 cm) and mower height (4.5 cm) following one (winter) or two (winter and spring) applications of N fertiliser (50 kg/ha) or gibberellic acid (GA; 8 g a.i./ha).

Nitrogen	0		N			*P* values		
Gibberellic acid	0	GA	0	GA	SEM[1]	GA	N	GA × N
Ground level								
One application	904	1309	1120	1528	75.4	<0.001	0.018	0.98
Two applications	2216	2252	3418	3289	150	0.76	<0.001	0.60
Combined yield	3121	3562	4538	4817	194	0.10	<0.001	0.67
Mower height								
One application	658	1142	920	1156	85	0.002	0.14	0.18
Two applications	1756[c]	2291[bc]	3010[a]	2551[ab]	184	0.84	0.003	0.02
Combined yield	2414[a]	3433[b]	3930[b]	3707[b]	181	0.06	<0.001	0.008

[1]SEM, standard error of the mean for the interaction.

GA. Although the colour differences eventually disappeared, the height effect of GA was still evident 33 days later when sward surface height was measured (Table 3). The botanical composition of the swards was dominated by perennial ryegrass which accounted for 830 ± 25 g/kg DM. There was a positive effect of GA on total ryegrass and white clover yields ($P < 0.05$), although, because perennial ryegrass DM yield also increased, there was no GA effect on the proportion of white clover (Table 3). Nitrogen fertiliser application reduced white clover proportion ($P < 0.05$) while increasing perennial ryegrass proportion (+68 g/kg DM, $P < 0.05$). Weed and dead material contributed to less than 50 g/kg DM and were not affected by treatment.

Apart from a 1.6 cm increase ($P < 0.05$) in sward height, two applications of GA had no effect on sward characteristics. On the other hand, two N fertiliser applications increased sward height by 3 cm, reduced white clover content and yield, and doubled the density of perennial ryegrass seed head compared to plots which didn't receive N (Table 3; $P < 0.05$). Tiller density was greater in N fertilised plots after two applications. There was no effect of GA on tiller density after two applications.

Tiller morphology

An interaction between GA and N for tiller density showed that after one application tiller density was not affected by GA application but N fertiliser application increased tiller density in the absence of GA. Tillers from one GA application were 11.2 mg DM heavier, and had longer leaves, longer pseudostem and truestem length ($P < 0.05$; Table 4). Nitrogen fertiliser increased leaf width by 0.32 mm compared with plots without N fertiliser. After two applications, the effect of GA on leaf and pseudostem length was no longer apparent (Table 4). There was an interaction between GA and N treatment for the leaf to stem ratio of vegetative tillers, but in this instance both GA and N improved leaf to stem ratio compared with plants that received no N or GA (Table 4). Plots that

Table 3 Sward characteristics of perennial ryegrass-based pastures following one (winter) or two (winter and spring) applications of N fertiliser (50 kg/ha) or gibberellic acid (GA; 8 g a.i./ha).

Nitrogen	0		N			*P* value		
Gibberellic acid	0	GA	0	GA	SEM[1]	GA	N	GA × N
One application								
Compressed height (cm)	3.55	5.9	3.75	5.6	0.26	<0.001	0.86	0.38
Clover (g/kg DM)	150	196	101	129	26	0.19	0.06	0.74
Clover yield (kg DM/ha)	138	253	111	188	31	0.01	0.18	0.56
Ryegrass (g/kg DM)	796	789	877	844	27	0.49	0.03	0.65
Ryegrass yield (kg DM/ha)	723	1036	983	1301	78.0	0.003	0.008	0.98
Ryegrass density[2] (tillers/m^2)	1892^b	2636^b	3798^a	2906^{ab}	355	0.84	0.01	0.05
Two applications								
Compressed height (cm)	9.6	11.55	12.95	14.15	0.63	0.03	0.001	0.55
Clover (g/kg DM)	299	372	164	140	27.1	0.39	<0.001	0.11
Clover yield (kg DM/ha)	658	841	559	455	65.6	0.57	0.005	0.06
Ryegrass (g/kg DM)	268	244	201	254	21.9	0.52	0.22	0.11
Ryegrass yield (kg DM/ha)	1313	1209	2281	2573	174.2	0.60	<0.001	0.29
Ryegrass stem (g/kg DM)	328	288	466	528	47.7	0.82	0.003	0.31
Stem density (stems/m^2)	798	705	1508	1527	184	0.84	0.002	0.77
Ryegrass density[2] (tillers/m^2)	2966	2609	4309	4658	390	0.99	0.002	0.39

[1]SEM, standard error of the mean for the interaction.
[2]Total vegetative and flowering ryegrass tillers.
Values in the same row with differing superscripts are significantly different ($P < 0.05$).

Table 4 Tiller morphology of perennial ryegrass following following one (winter) or two (winter and spring) applications of N fertiliser (50 kg/ha) or gibberellic acid (GA; 8 g a.i./ha).

Nitrogen	0		N			*P* value		
Gibberellic acid	0	GA	0	GA	SEM[1]	GA	N	GA × N
One application								
Leaf length (mm)	86	124	91	135	4.59	<0.001	0.09	0.56
Leaf width (mm)	3.14	3.28	3.48	3.58	0.08	0.18	0.004	0.88
True stem length (mm)	0.44	4.79	1.93	6.45	1.01	0.003	0.18	0.94
Pseudostem length (mm)	31.4	42.1	33.3	44.6	1.8	<0.001	0.25	0.86
Tiller mass (mg DM/tiller)	39.2[ab]	39.9[ab]	26.2[b]	48.0[a]	4.73	0.04	0.62	0.05
Leaf:stem ratio[2] (g/g DM)	2.21[b]	2.19[b]	3.34[a]	2.01[b]	0.17	0.003	0.02	0.003
Two applications								
Leaf length (mm)	107	127	128	141	8.3	0.08	0.06	0.67
Leaf width (mm)	2.75	2.5	2.6	2.63	0.06	0.1	0.87	0.06
Pseudostem length (mm)	32.5	29.7	35.7	31.6	3.16	0.31	0.44	0.85
Tiller mass (mg DM/tiller)	25.1	26.7	23.5	25.2	1.88	0.4	0.45	0.98
Leaf:stem ratio[2] (g/g DM)	2.25[b]	3.14[a]	2.94[a]	2.98[a]	0.16	0.02	0.13	0.02
Flag leaf length (mm)	90.2	98.6	101.3	110.5	5.86	0.14	0.09	0.46
True stem length (mm)	124	134	154	157	8.99	0.39	0.01	0.66
Stem mass (mg DM/stem)	88.6	83.8	100.1	109.6	9.97	0.82	0.09	0.49

[1]SEM, standard error of the mean for the interaction.
[2]Leaf to stem ratios are for vegetative tillers.
Values in the same row with differing superscripts are significantly different ($P < 0.05$). Measurements determined from tillers to ground level.

received N fertiliser had longer seed heads ($P < 0.05$) and tended to have larger flowering tillers than non N treated plots (105 vs 86 mg DM/tiller, $P < 0.10$).

Nutritive composition

Gibberellic acid altered nutrient composition of perennial ryegrass after one application by increasing fibre content (both NDF and ADF fractions) and reducing crude protein content (Table 5). This was accompanied by a small but significant reduction in digestibility of 1.2% ($P < 0.05$) in GA plots compared with non-GA plots. Average CP content was relatively high at 225 g CP/kg DM in the first harvest. Nitrogen fertiliser increased the CP by 19% ($P < 0.05$), reduced NDF by 4% and reduced SSS by 10% compared to perennial ryegrass with no N fertiliser ($P < 0.05$). Following two applications of GA, fibre content was reduced ($P < 0.05$) but minor changes in CP and SSS were not significant and there was no effect of GA on DOMD.

Nitrogen fertiliser increased Cu, K and Zn concentrations and reduced the Cd concentration in perennial ryegrass (Table 6). An interaction between GA and N for B showed the B concentration was increased by N fertiliser but not when GA was applied. Gibberellic acid had little affect on mineral concentration. Sodium concentration was increased by 47% by GA application. The milliequivalent ratio of K/Ca + Mg was 1.34 ± 0.07 and it was unaffected by one GA or N application.

Discussion

DM yield reponse after one application

Irrespective of whether N fertiliser was applied, the immediate response to an application of 8 g a.i./ha of GA in late winter was a 40% increase in DM yield above ground level in

Table 5 Nutritive composition (g/kg DM) in the perennial ryegrass portion of pasture following one (winter) or two (winter and spring) applications of N fertiliser (50 kg/ha) or gibberellic acid (GA; 8 g a.i./ha).

Nitrogen	0		N			*P* value		
Gibberellic acid	0	GA	0	GA	SEM[1]	GA	N	N × GA
One application								
Organic matter	917	918	913	916	1.0	0.08	0.01	0.18
Acid detergent fibre	149	161	141	155	1.5	<0.001	0.002	0.36
Neutral detergent fibre	295	318	280	309	2.7	<0.001	0.002	0.27
Soluble sugars and starch	330	339	301	300	6.6	0.61	0.001	0.44
Protein	217	193	251	238	5.2	0.006	<0.001	0.30
DOMD[2]	887	876	886	873	1.7	<0.001	0.19	0.52
Two applications								
Organic matter	907	906	909	907	2.3	0.65	0.62	0.81
Acid detergent fibre	215	203	211	203	4.2	0.04	0.70	0.62
Neutral detergent fibre	394	370	390	380	6.6	0.03	0.65	0.33
Soluble sugars and starch	329	355	287	296	8.2	0.07	<0.001	0.33
Protein	130	135	163	166	6.9	0.59	0.001	0.95
DOMD[2]	809	821	801	807	5.9	0.15	0.10	0.60

[1]SEM, standard error of the mean for the interaction.
[2]DOMD, digestible organic matter in the dry matter (g/kg).

early spring. This is equivalent to an additional 51 kg DM/g GA (Table 2). Recent studies comparing low rates of GA (between 8 and 30 g a.i./ha) on perennial ryegrass based pastures have also reported positive responses to GA both above mowing height (32% increase, 20 kg DM/g GA; Zaman et al. 2014) and above ground level (17% increase, 24 kg DM/g GA; Matthew et al. 2009). In an independent assessment of a commercial product containing GA, Edmeades & McBride (2012) found response to ProGibb at 8 g a.i./ha ranged between 13% and 63% with a mean DM yield increase of 36%. Similarly, in their study of pasture responses to fertiliser products Edmeades & McBride (2012) noted that responses to N were always positive, with a mean DM yield increase of 57% and a response range of 10% to 145%.

Table 6 Mineral composition (mg/kg DM unless otherwise indicated) of perennial ryegrass following one (winter) application of N fertiliser (50 kg/ha) or gibberellic acid (GA; 8 g a.i./ha).

Nitrogen	0		N			*P* value		
Gibberellic acid	0	GA	0	GA	SEM[1]	GA	N	N × GA
B	6.6[c]	7.6[b]	10.1[a]	7.2[b]	0.18	<0.001	<0.001	<0.001
Ca	4727	4946	4978	4890	125	0.61	0.46	0.26
Cd	0.02	0.02	0.03	0.02	0.033	0.60	0.02	0.18
Co	0.03	0.03	0.04	0.04	0.003	0.63	0.27	0.60
Cr	0.159	0.150	0.160	0.149	0.008	0.24	0.98	0.86
Cu	3.04	3.23	3.45	3.64	0.15	0.23	0.02	0.99
Fe	98.4	98.0	126.3	107.5	11.94	0.44	0.16	0.46
K	19499	18235	21600	22810	802	0.97	0.003	0.16
Mg	1770	1862	1883	1896	59.5	0.41	0.25	0.53
Mn	78.1	75.0	73.9	70.5	6.38	0.62	0.51	0.98
Mo	0.126	0.108	0.128	0.09	0.033	0.42	0.81	0.77
Na	3070	4180	3088	4676	321	0.003	0.45	0.48
P	2514	2493	2571	2552	69.5	0.78	0.43	0.99
S	2323	2323	2419	2480	63.5	0.65	0.08	0.65
Zn	17.2	18.4	22.68	25.0	1.70	0.33	0.007	0.75

[1]SEM, standard error of the mean for the interaction.

In the current study, the DM yield response of pasture to one application of N fertiliser was low at 4.3 kg DM/kg N. In grasses such as perennial ryegrass DM yield is a function of both tiller number and size, and grasses will often compensate one or the other to achieve maximum leaf area index (Bircham & Hodgson 1983; Matthew et al. 1995). Response of pastures to N fertiliser in spring typically follows this growth strategy, often exhibiting increased tillering and tiller density (Gislum & Griffith 2004), wider, longer leaves and lower weight per unit area (Wilman & Pearse 1984). Over time the regrowth cycle switches to one of self-thinning due to canopy closure and shading of the tiller base if defoliation does not occur. In the current study, the low thermal accumulation in the first regrowth cycle (175 GDD) supports observations for short term N response of increased leaf width and tiller density. Because leaf tissue expansion is driven by temperature and N when water is not limiting (Gastal et al. 1992), it is likely that temperature, rather than N, limited the response to N fertiliser in the first regrowth cycle.

Nitrogen fertiliser is also often responsible for the suppression in clover content in mixed swards (Harris et al. 1996), generally due to competition. The increased tiller density of perennial ryegrass and subsequent dominance of botanical content indicate that the positive DM yield response to N at ground level was due to the perennial ryegrass rather than the white clover. This could explain the lack of N effect on total yield above mowing height as the recent initiation of tillers were not sufficiently large to contribute to yield above 4.5 cm (Table 2).

On the other hand, the positive pasture DM yield response to GA was attributed to both the increase in white clover yield in addition to perennial ryegrass yield. The increased white clover yield in mixed swards in response to GA observed in this study agrees with previous observations (Percival 1980; van Rossum et al. 2013). Matthew et al. (2009), in their review on gibberellins, highlighted the range of functions gibberellins play in plant growth and that the size of the response to exogenous GA is often influenced by the plant species to which it is applied. Moreover, greater sensitivity of white clover compared with grass was demonstrated in a pot study by Finn & Nielsen (1959) who found that legumes, including white clover, were more responsive to GA than grasses as clover yields continued to increase with increasing rate of GA application.

The presence of ryegrass yield response to GA was intuitive as there were visible differences in height of tillers between plots which had or had not received this treatment. Typically, grasses respond to GA by elongation of leaf and sheath tissues, manifesting in increased height (Matthew et al. 2009) which is consistent with our own observations of increased leaf and stem and greater compressed height (Tables 3 and 4). Earlier studies have also reported significant ryegrass response to GA (Morgan & Mees 1958; Finn & Nielsen 1959; Zaman et al. 2014). However, these experiments used much higher rates of between 20 and 600 g a.i./ha compared to the 8 g a.i./ha used in the current study.

An interaction between GA and N in the present study showed that both tiller size and density were improved when GA-treated plants also received N. A recent controlled environment pot study by Parsons et al. (2013) examined the DM response of winter-derived perennial ryegrass after 2, 3 or 4 weeks regrowth using low rates of GA (8 g a.i./ha) comparable to that used in the current study. In comparison, the yield results of Parsons et al. (2013) after 3 weeks' regrowth, which are equivalent in thermal time units (178 GDD) to those of the current study (175 GDD), showed little aboveground response of GA-treated ryegrass under low N supply. Under high N supply, however, Parsons et al. (2013) showed significant increases in shoot DM yield. Further field studies which include root measurements would aid understanding of biomass allocation as the aforementioned pot study indicates some important growth responses to GA under nutrient-limiting conditions.

By shifting the distribution of tiller mass to leaf, sheath and stem, the resulting GA-treated pasture would have had a bulk density which favoured the upper sward horizon. While this may not have given way to increased perennial ryegrass DM yield at ground level, the significant effect of GA relative to N above mowing height suggests that perennial ryegrass is likely to have been an important contributor to total DM yield, when determined above mowing height (Table 3). The practical implications of assessing DM yield to ground level or grazing height are complex, as on the one hand increased bulk density at the base of the sward through increased tillering has positive correlations with persistence and long term pasture yield (Edwards & Chapman 2011), while on the other hand it is favourable to increase sward height and bulk density to promote DM intake (Laca et al. 1992). Tools which measure herbage mass—such as the rising plate meter—for feed planning and animal allocation purposes, are sensitive to variation in sward bulk density and alternative calibrations are likely to be required when used to determine pasture mass of GA-treated pastures.

DM yield response after two applications

There was no improvement in pasture DM yield when two consecutive GA applications occurred (Table 2), and evidence that yield reductions may be possible was shown by the negative DM yield response evident after the second application (–6 kg DM/g GA). There is little reported data on repeat low rate applications of GA, although several studies note DM yield depressions in subsequent harvests when no GA or N is applied consecutively (Morgan & Mees 1958; Leben et al. 1959). Those authors attributed the yield depression to reduced access to soil nutrients through depletion of root stocks. Morgan & Mees (1958), however, tested this by replacing N and other minerals following the first harvest and found this had little effect on subsequent DM yield depression as a result of GA application. Morgan (1968) later postulated that elongation of stem in GA plants led to removal of a larger proportion of leaf area after cutting and slower relative growth rate. Loss of leaf area after cutting and insufficient regrowth interval before the following harvest, which would allow plants to replenish depleted reserves, may both contribute to long term reductions in growth from GA-treated plants. In the current study lower application rates (8 g a.i./ha) and lengthy regrowth intervals of 5 weeks may have allowed pastures to recover sufficiently from loss of leaf area and any loss of stubble or root reserves—a response which appears evident in the initial stages of growth (first 2 weeks) as shown by Parsons et al. (2013).

In this study the cost of applying GA, and the DM response obtained, demonstrate that cost is not expected to prevent farmer adoption. At current retail prices of $188/250 g ProGibb and $575/tonne urea the cost of extra DM grown in this experiment was equivalent to 4 c/kg DM or 29 c/kg DM following one application of GA or N respectively or 7 c/kg DM or 9 c/kg DM for net DM yield following two applications of GA or N respectively. Economically, use of GA to increase pasture production following one or two consecutive applications is considerably lower than estimated break-even feed costs of < 20c/kg DM reported by Laborde et al. (1998).

Nutritive composition

Any improvement in DM yield in response to GA should not occur at the expense of the nutritional value of the herbage. As shown above, treatment of pastures with GA resulted in elongation of leaf, sheath and increased stem of ryegrass. This led to increased fibre and numerically lower digestibility (DOMD) of ryegrass, though it could also be argued that digestibility values of over 80% reflect potential for high energy supply. There was little effect of N fertiliser on organic matter digestibility, which supports observations of Peyraud & Astigarraga (1998) who reviewed the effects of N fertiliser on nutritive value and found only a small positive effect (0.02 units) of N on digestibility. Those authors noted that the variation in DOMD was related to changes in cell wall content which was likely to decline following N fertilisation in early regrowth when growth was succulent, but the effects of N on cell wall disappeared after 6 weeks. In the present study, considering the low thermal time and reduced fibre content due to N treatment, our results are consistent with the effects of N on cell wall in early regrowth.

An important finding as a result of GA treatments was the negative effect on crude protein (CP) concentration of the ryegrass. With one application of GA, increased DM yield was achieved at a lower herbage N content than the control, whereas with one application of N fertiliser, increased DM yield was achieved with a higher herbage N content than the control. In earlier studies, which included measurements of herbage N or protein concentrations, reductions in N concentration in GA treated plants were observed in some (Morgan & Mees 1958; Finn & Nielsen 1959; Percival 1980), but not all (Scott 1959; Matthew et al. 2009; Parsons et al. 2013) experiments. Explanation for the variation in protein response of plants treated with GA is unclear, but may be linked to a combination of influences due to the environment and regrowth duration. These results may have important implications for management of N losses (eg. nitrate and nitrous oxide) from animals in livestock grazing systems as mineral N concentration in the forage and dietary N intake are closely linked to drivers of N loss (Higgs et al. 2012; Whitehead & Edwards 2015). However, it is important to note here that GA application did result in increased white clover content which may offset reductions in ryegrass N concentration, due to white clover which typically has a herbage N concentration of 40–48 g/kg DM (Ayres et al. 1998; Black et al. 2007).

Gibberellic acid had little impact on the concentration of minerals in ryegrass, with the one exception being Na uptake which was increased by over 1.4 g Na/kg DM compared to non-GA treatments. The reasons for this are not clear, though increased Na in GA-treated pasture could be associated with increased water demand from stimulated growth, increase in cell expansion and cell volume, and changes in sugar transport, which are all regulated in some way by GA (Paleg 1965). The limited effect of GA on minerals in the present study support those of Morgan & Mees (1958) who observed no changes in mineral uptake of perennial ryegrass pastures as a result of GA treatment. The increased K concentration of herbage due to N fertiliser supports earlier observations (Kemp 1983; Mckenzie & Jacobs 2002). The ratio of K/(Mg + Ca) was not affected by GA application and was below the recommended maximum of 2.2 for reducing the risk of hypomagnesemia (Kemp & t'Hart 1957; Grunes & Welch 1989).

Conclusion

In late winter, one application of GA, at a rate of 8 g a.i./ha, resulted in increased shoot DM yield, although there was no apparent benefit in applying two applications of GA. Nitrogen fertiliser also increased DM yield with ongoing DM yield advantages following two applications. In binary pasture mixtures, such as that of perennial ryegrass and white clover, DM yield is determined by the relative growth of each species and by the size and density of the plant units. While the effect of one application of GA or N on DM yield were positive, the factors determining the yield differed between treatments and this has practical implications for determination of DM yield and nutritive value. One application of GA in late winter could be used to reduce herbage CP content and reduce the risk of urinary N losses; however, these hypotheses require testing under grazing as differences in clover may maintain high N intake. The use of GA in conjunction with N fertiliser to promote maximum pasture production in early spring is recommended as a tool for farming systems to reduce early season feed shortages.

Disclosure statement

No potential conflict of interest was reported by the authors.

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