The effect of sulphur and nitrogen fertiliser on levels of anti-nutritional compounds in kale

A.L. FLETCHER[1], D.R. WILSON[1], S. MALEY[1], J. McCALLUM[1] and M. SHAW
[1]*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704 Christchurch, 8140*
Andrew.Fletcher@plantandfood.co.nz

Abstract

Kale is used throughout New Zealand as a supplement to pasture during winter. However, kale contains the anti-nutritional compounds S-methylcysteine sulphoxide (SMCO), glucosinolates and nitrate (NO_3^-). All can be increased by inappropriate applications of fertiliser nitrogen (N) and sulphur (S). When concentrations exceed either 1.5% SMCO or 2 000 mg NO_3^-/kg DM in forage, the performance of grazing animals may be reduced. Experiments were undertaken in 2005/2006 at Hinds (moderate soil N and S) and Lincoln (high soil N and S) to test the effect of four rates of N (0, 100, 200 and 300 kg/ha) and three rates of S (0, 50 and 100 kg/ha) fertiliser on yield, SMCO, NO_3^- and glucosinolate content in kale. Sulphur fertiliser did not increase yield at either site while N only increased yield at Hinds. At Hinds, regardless of fertiliser S or N, the SMCO contents of forage were below 1.5% of DM and unlikely to affect grazing animals. At Lincoln, fertiliser N increased SMCO, but S did not. At the highest rates of N the SMCO contents were above the 1.5% risk threshold and would likely have affected animal performance. Glucosinolates were higher at Lincoln than at Hinds, reflecting the higher background soil S at Lincoln. Fertiliser had only a minor effect on glucosinolate levels. Leaf NO_3^- content was increased by fertiliser N at both sites, but was always below the risk threshold. Fertiliser N should be carefully managed in kale crops to avoid the build up of potentially harmful SMCO and NO_3^- levels, particularly on sites with high soil S.

Keywords: *Brassica*, glucosinolate, nitrate, S-methyl cysteine sulphoxide

Introduction

Kale (*Brassica oleracea* var. *acephala*) is used as a winter forage crop to supplement pasture throughout New Zealand. A well managed kale crop can exceed 20 t DM/ha of high quality forage (Zyskowski *et al.* 2004; Wilson *et al.* 2006; Brown *et al.* 2007; Fletcher *et al.* 2007). However, like all brassicas, it produces several secondary compounds that can markedly reduce animal performance (Nichol 2007; de Ruiter *et al.* 2009), including S-methyl cysteine sulphoxide (SMCO), nitrate (NO_3^-) and glucosinolates.

SMCO is an amino acid that contains N and S (Nichol 2007). At low concentrations there may be no apparent effects on animals, but at moderate concentrations they may reduce feed intake and animal growth rate. At high levels these compounds may cause anaemia (extreme cases result in death). SMCO concentrations generally vary between 0.6 and 2.0%. There is no established critical value for SMCO content in kale. However, when Barry *et al.* (1982) feed kale supplemented with SMCO to lambs, liveweight gain decreased with each increase in SMCO but there was a marked decrease when SMCO concentration exceeded 1.5%. Thus, throughout this paper 1.5% is considered the risky concentration for animal performance. Kale has higher levels of SMCO than other forage brassicas (Gowers & Nicol 1989). Stage of plant development influences SMCO content with flowering plants generally having higher concentrations of SMCO than vegetative plants (Gowers & Nicol 1989). The application of fertilisers that contain both N and S can increase SMCO levels in brassicas, depending on soil fertility (especially S levels) (Barry *et al.* 1982).

NO_3^- concentrations in brassicas can be higher than in other forages, are dynamic and can change quickly in the plant. For example, environmental conditions such as cool, dull weather increase NO_3^- concentrations (Charlton & Stewart 2000; Nichol 2007). Kale is utilised during the winter so is often fed to stock in cool, dull conditions. This increases the risk of elevated NO_3^- levels in these crops because NO_3^- continues to be assimilated but reduced photosynthesis means that it is not converted into amino acids. Furthermore, excessive soil N supply increases the risk of high NO_3^- content in forage. At normal concentrations (<1 000 mg/kg) NO_3^- poses few problems to animal health. However, when concentrations exceed ~2 000 mg/kg (Charlton & Stewart 2000; Nichol 2007), NO_3^- interferes with oxygen binding to haemoglobin in the blood and, in severe cases, causes rapid animal death.

Glucosinolates are a group of S-containing compounds that function as a plant defence mechanism against pests and pathogens (Falk *et al.* 2007). High levels of glucosinolate interfere with iodine metabolism and can cause goitre (Nichol 2007) in animals. Glucosinolates also reduce the palatability of feed (Gustine & Jung 1985). Gustine & Jung (1985) also found that glucosinolate concentrations in a range of forage brassicas (including kale) were much higher than the 3 g/kg level that commonly affects animal performance. Glucosinolates contain both N and S and therefore their concentrations in forage are influenced by the addition of N and S fertiliser (Falk *et al.* 2007).

Although it is likely that N and S fertiliser applications will increase the content of these three compounds in kale (McDonald *et al.* 1981; Falk *et al.* 2007), adequate mineral nutrition is also essential for high yielding brassica crops (Wilson *et al.* 2006). Forage brassicas often respond strongly to N fertiliser but seldom to S (Wilson *et al.* 2006). The response to N will depend on background soil N. For example, Fletcher *et al.* (2007) found no yield response of kale to N fertiliser on a high N site, but marked yield increases on a low N site. At the high N site the additional N fertiliser markedly increased crop N accumulation. This could potentially have increased the levels of SMCO, glucosinolates and NO_3^- in forage. An assessment of how soil and fertiliser sources of N and S affect these compounds is necessary to manage these potential tradeoffs between increased yield and increased anti-nutritional compounds.

Although many contributing factors to high SMCO, NO_3^- and glucosinolate levels in brassicas have been identified, the degree to which N and S supply affects these concentrations has not been widely examined in New Zealand. This paper reports the effects of a range of N and S fertiliser treatments on SMCO, NO_3^-, and glucosinolate concentrations in kale leaves in two experiments at Hinds (a site with moderate soil S) and Lincoln (a site with high soil S) in Canterbury, New Zealand.

Methods

Sites

The Hinds experiment (44.0° S, 171.6° E) was sown on 25 November 2005 on a stony Lismore soil. Soil samples taken to 150 mm depth on the same day showed

Figure 1 S-methyl cysteine sulphoxide (SMCO) concentration of kale leaves in response to nitrogen (N) and sulphur (S) fertiliser applications at (a) Hinds on 3 May (b) Hinds on 31 May (c) Lincoln on 17 May and (d) Lincoln on 15 June 2006. The dotted line represents the concentration considered risky for animal health. Error bars represent the LSD ($\alpha < 0.05$; 22 d.f.) for comparing N treatments.

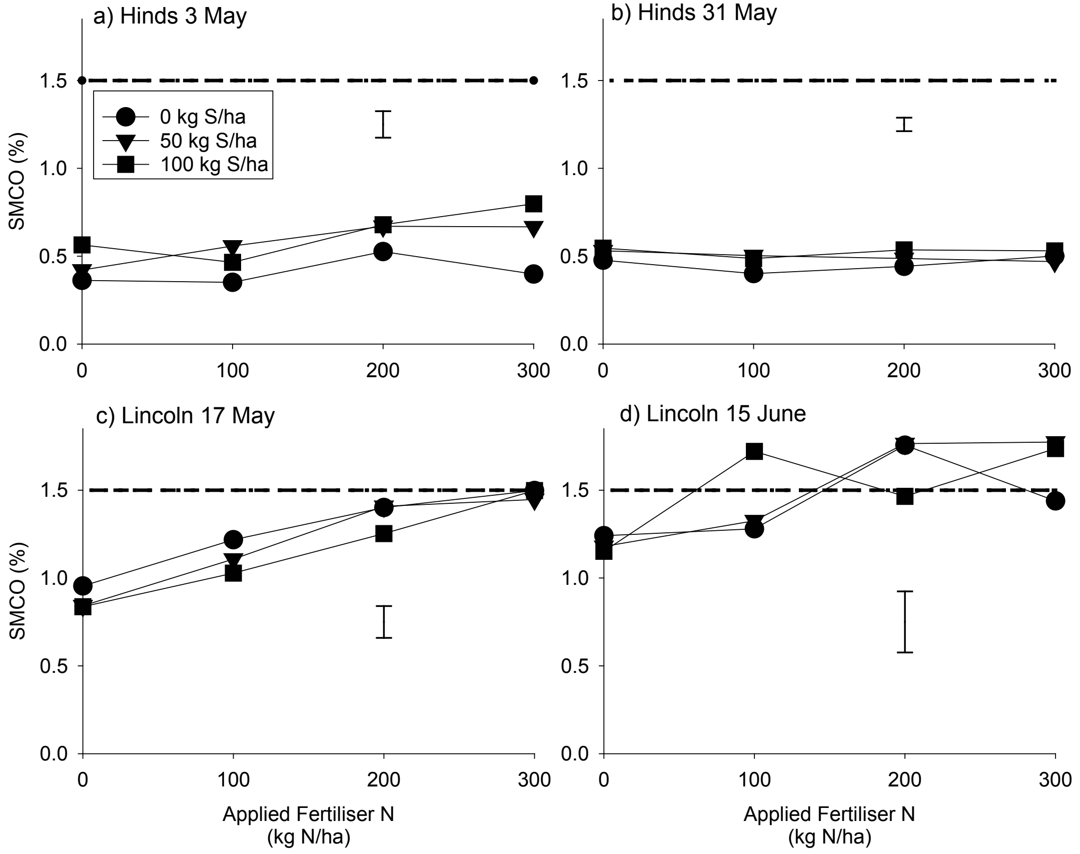


Table 1 Effect of nitrogen (N) and sulphur (S) fertiliser on whole crop dry matter yields (t DM/ha) of kale.

Fertiliser applied	0 kg N/ha	100 kg N/ha	200 kg N/ha	300 kg N/ha	Mean
Lincoln, 17 May					
0 kg S/ha	16.9	17.0	19.7	18.1	17.9
50 kg S/ha	18.1	19.0	17.2	19.0	18.3
100 kg S/ha	17.0	16.7	19.3	19.4	18.1
Mean	17.3	17.6	18.7	18.8	18.1
LSD (N)	2.0				
LSD (S)	1.7				
LSD (N×S)	3.4				
Hinds, 3 May					
0 kg S/ha	13.5	16.9	18.5	18.0	16.7
50 kg S/ha	11.1	18.2	16.9	17.5	15.9
100 kg S/ha	12.7	15.0	17.3	19.9	16.2
Mean	12.4	16.7	17.6	18.5	16.3
LSD (N)	2.1				
LSD (S)	1.8				
LSD (N×S)	3.6				
Hinds, 31 May					
0 kg S/ha	14.3	16.7	18.5	20.1	17.4
50 kg S/ha	12.7	15.3	17.8	17.9	15.9
100 kg S/ha	13.8	17.2	20.1	19.8	17.7
Mean	13.6	16.4	18.8	19.3	17.0
LSD (N)	2.3				
LSD (S)	2.0				
LSD (N×S)	3.9				

[1] Least significant difference ($\alpha<0.05$; 22 d.f.) for comparing nitrogen treatment main effects.

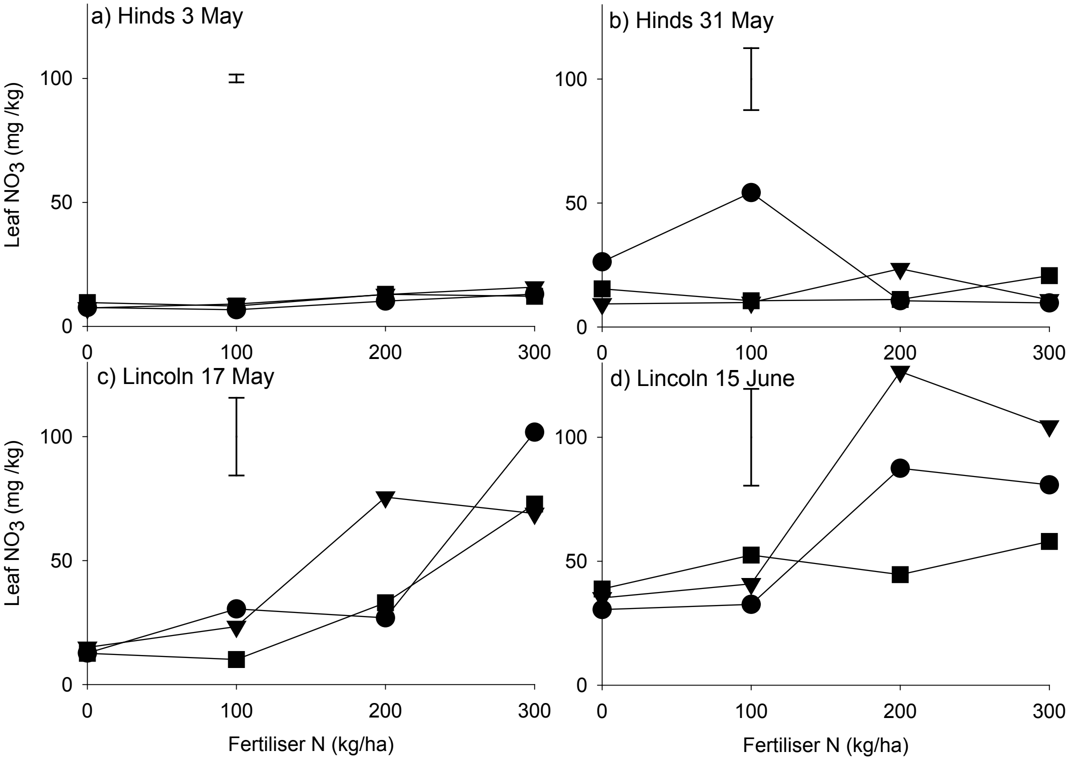
a medium, readily available N content (123 kg/ha), a soil sulphate (SO_4^{-2}) level (5 ppm), a pH of 6.2, and a moderate Olsen P (16 µg/ml). The Lincoln experiment (43.6° S, 172.5° E) was sown on 30 November 2005 on a Templeton silt loam soil. Soil samples taken to 150 mm depth on the same day showed a low, readily available N (58 kg/ha), a high soil SO_4 level (19 ppm), a pH of 5.8, and a high Olsen P (27 µg/ml). The readily available N test uses an anaerobic incubation (Keeney & Bremner 1966) to indicate the potential of a cultivated soil to mineralise N. At Lincoln, deeper soil tests showed that there was also considerable mineral N (257 kg N/ha) to a depth of 1 m.

Experimental design and management

An identical treatment structure was used at both sites. This consisted of a complete factorial combination of three rates of S fertiliser (0, 50 and 100 kg S/ha) applied as gypsum (18% S) and four rates of N fertiliser (0, 100, 200, and 300 kg N/ha) applied as urea (46% N) laid out in a randomised complete block design with three replicates. At both sites kale cv. 'Gruner' was sown at 3.5 kg/ha using a plot seeder. Plots (1.65 x 15 m) contained 11 rows spaced 150 mm apart. Base fertiliser was broadcast onto both sites at 41 kg P/ha (as triple super phosphate), 35 kg K/ha (applied as KCl), and 1.5 kg B/ha (applied as boronate; 10% B), immediately before sowing. The S treatments were broadcast by hand at sowing. The N treatments were broadcast by hand in three split applications; one-third applied at sowing, 4 January, and on 4 February for both experiments.

The crops at both sites were irrigated with overhead irrigation to replace evapo-transpiration. Weeds and insect pests were controlled using a combination of herbicide and pesticide sprays. Just before sowing, 2 l/ha of Treflan was applied and incorporated at both sites to control weeds. Also at sowing, 350 ml/ha of Diazinon was applied to control springtails and thrips. On 16 December, Lorsban at 1 L/ha was applied at both sites to prophylactically control a range of insects. A further application of Lorsban at 600 ml/ha was made on 4 January. Radiate was applied at the Lincoln site on 29 December to control volunteer potatoes. On 26 and 27 January Karate was applied at the Lincoln and Hinds sites, respectively, at 40 ml/ha to control white butterfly.

Figure 2 Leaf NO_3^- concentration of kale in response to nitrogen (N) and sulphur (S) fertiliser application at (a) Hinds on 3 May (b) Hinds on 31 May (c) Lincoln on 17 May and (d) Lincoln on 15 June 2006. Error bars represent the LSD ($\alpha < 0.05$; 22 d.f.) for comparing N treatments. Symbols as in Figure 1.



Measurements

At Hinds crop biomass was measured on 3 and 31 May 2006. At each harvest, leaf samples were also collected for SMCO, NO_3^-, and glucosinolate determination. At Lincoln crop biomass was measured on 17 May only and SMCO, NO_3^-, and glucosinolate concentrations were determined on samples taken on 17 May and 15 June. Biomass was not measured on 15 June because a major snowfall on 12 June caused major crop damage and leaf loss, making an accurate yield assessment impossible.

Crop biomass was estimated by cutting a 3 m^2 quadrat sample to ground level. This sample was weighed fresh and then a 10 plant sub-sample was randomly selected to determine dry matter (DM) content. These sub-samples were used to calculate crop DM yield for each plot and were split into stem and leaf fractions.

At each harvest SMCO, NO_3^-, and glucosinolate concentrations were determined on the last fully expanded leaf collected from 20 randomly selected plants per plot. A 3 cm wide strip was taken from across the centre of each leaf. These were frozen rapidly and stored at -20°C before freeze-drying. The SMCO content was determined by a spectrophotometric method adapted from Gosden (1979). The NO_3^- content was determined by the method of McCallum *et al.* (2005). The content of two glucosinolates was determined indirectly by measuring the content of two isothiocyanates (ITC; the volatiles formed from glucosinolates) using the method of Sultana *et al.* (2003).

All data were analysed using analysis of variance with a measure of the variation associated with predicted means based on Fischer's protected least significant difference (LSD) ($\alpha = 0.05$).

Results and Discussion

Fertiliser N or S application had no effect on crop DM yield for the single harvest at Lincoln (Table 1). Overall, the mean yield was 18.1 t/ha. In contrast, N applications increased DM yields but S applications had no effect for both harvests dates at Hinds (Table 1). Applying 100 kg N/ha gave the greatest increase in yield. Applying 200 kg N/ha gave a further small, but significant increase in yield and applying 300 kg N/ha gave no further significant yield increase. Both sites had high potential yields of between 18 and 20 t DM/ha (Table 1) and therefore high demand for N (Wilson *et al.* 2006). A 20 t DM/ha kale crop would require approximately 400 kg N/ha. At Lincoln, the 257 kg N/ha (of mineral N) in the top 1 m of soil at sowing supplied most of this requirement and with even a modest amount of N mineralisation the lack of response is not surprising. In contrast at Hinds there was less mineral N at depth and the mineralisation of N was insufficient to meet crop N requirement resulting in a moderate response to N fertiliser (Table 1). The lack of response to S at both sites was also expected (Wilson *et al.* 2006).

At Hinds (low S site) all SMCO concentrations were well below the 'risky' threshold value of 1.5% of DM (Figs. 1a, b). Applying both N and S fertiliser increased ($P<0.05$) the SMCO concentration of leaves harvested on 3 May (Fig. 1a). Without added S, the mean SMCO content was 0.41% of DM, but when 100 kg S/ha was added the mean SMCO concentration was 0.63%. Without added fertiliser N, the mean leaf SMCO concentration was 0.45% of DM, which increased to 0.62% when 200 or 300 kg N/ha was applied. There were no significant effects of N or S fertiliser application on leaf SMCO concentrations at Hinds for the 31 May harvest (Fig. 1b). It is not clear why the significant effect of S fertiliser on SMCO was apparent at 3 May but not 31 May.

At Lincoln (high soil S), SMCO concentrations were much higher than at Hinds, and in some cases they were above the 'risky' threshold value of 1.5% (Figs. 1c, d). For both harvest dates, SMCO content increased with increasing amount of N fertiliser, but was unaffected by S fertiliser. At the 17 May harvest, mean leaf SMCO concentration increased ($P<0.001$) from 0.88% of DM with no N fertiliser to 1.48% with 300 kg N/ha applied. At the 15 June harvest, mean leaf SMCO concentration increased ($P<0.05$) from 1.19% of DM with no N fertiliser to 1.65% with 300 kg N/ha applied. The clear implication of these results is that when the soil S level is high, growers should take care not to over-apply N fertiliser because it is likely to increase the SMCO content of kale. This confirms the findings of McDonald (1982) and McDonald *et al.* (1981). The effects of SMCO on grazing animal performance are dependent on the total intake of SMCO (Nichol 2007). Therefore, the risk of SMCO poisoning can be limited by feeding high fibre supplements such as hay or straw before feeding kale. When soil S is low, applying S fertiliser may increase SMCO content, but SMCO levels are likely to remain well below the level at which animal performance is reduced.

Site had the greatest influence on glucosinolate concentrations, being much higher at Lincoln than at Hinds for both of the glucosinolates measured. Mean total ITC content was 6.1 µmol/g DW at Lincoln and 3.3 µmol/g DW at Hinds (data not shown). This reflected the higher background soil S at Lincoln compared with Hinds. There was a significant increase in 3-butenyl ITC (the volatiles formed from gluconapin) following S fertiliser application at Lincoln but not at Hinds. This is consistent with the results of Rosen *et al.* (2005) who found that S fertiliser increased glucosinolate concentration of cabbage, but N fertiliser decreased glucosinolate concentrations. However, McDonald *et al.* (1981) found no increase in kale glucosinolate concentration with S fertiliser, at either a low or a high fertility site, but a decrease in glucosinolate concentration when N fertiliser was added.

At both Hinds and Lincoln all leaf NO_3^- concentrations were between 10 and 120 mg/kg (Fig. 2), which is well below the 'risky' threshold value of 2 000 mg/kg (Charlton & Stewart 2000; Nichol 2007) and therefore there was negligible risk for animal health. At the first harvest at Hinds and for both harvests at Lincoln, applying fertiliser N significantly increased the NO_3^- concentration of the leaves, but applying S fertiliser had no effect at either site. For the 3 May harvest at Hinds, leaf NO_3^- concentrations in the treatments with 0 and 100 kg N/ha applied were ~8 mg/kg averaged across all S fertiliser treatments. The values were 12.0 and 13.7 mg/kg with 200 and 300 kg N/ha applied, respectively. Leaf NO_3^- concentrations were much higher at Lincoln, probably due to the substantial amount of mineral N available in the soil below 150 mm depth. However, the concentrations were still well below the 2 000 mg/kg risk threshold value. For the 17 May harvest the NO_3^- concentration of leaves in the treatments with N fertiliser applied was significantly higher than when no N was applied ($P<0.001$). Averaged across all S fertiliser treatments, there was no significant difference between the 0 and 100 kg N/ha treatments, which had NO_3^- concentrations of 13.4 and 21.4 mg/kg, respectively (Fig. 2c). When 200 or 300 kg N/ha was applied, the leaf NO_3^- concentrations were 45.2 and 81.2 mg/kg, respectively. For the 15 June harvest at Lincoln, mean leaf NO_3^- concentrations were ~38 mg/kg for the 0 and 100 kg/ha N treatments (Fig. 2d). The values were significantly higher (~84 mg/kg; $P<0.05$) when 200 or 300 kg N/ha was applied. This suggests that if even higher rates of N fertiliser were applied there might have been further increases in NO_3^- concentrations. NO_3^- concentrations in these experiments were low, probably because of the prevailing weather conditions. In all forages, high NO_3^- levels most often occur in cool and dull conditions (Nichol 2007). A combination of these conditions and excessive N fertiliser application may push forage NO_3^- concentrations above 2 000 mg/kg, risking animal health. At these high levels of NO_3^- the risks of animal poisoning can be minimised by feeding a high fibre supplement such as hay or straw.

Conclusions

Fertilisers are applied to kale to increase yield when soil nutrients are limiting. However, care needs to be taken that excess fertiliser (especially N) is not applied. Excessive N applications will increase the risk of adverse animal health effects due to an increase in the NO_3^- and SMCO (especially on high S soils) content of kale.

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