Can nitrogen-fertilised ryegrass substitute for white clover?

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Abstract
Sheep and cattle have difficulty satisfying their preference for white clover when its proportion in pastures is low. We tested the hypothesis that they prefer clover because it has a higher concentration of nitrogen (N), and expected that they would reduce their preference for clover (increase their preference for grass) when grass had a higher concentration of N. In two experiments, mature sheep and growing cattle were offered choices between grass, having either a high or a low concentration of N, and white clover, growing as adjacent pure swards. To test the specific role of N in preference they were also offered each grass alone (sheep only) and a choice between the high and low N grass. Sheep and cattle preferred clover (75% of time grazing on clover and 25% on grass), but this preference was not affected by the concentration of N in the grass. They preferred the grass with a high concentration of N to that with a low concentration. Sheep and cattle detect differences in the N concentration of food items, but alter their grazing behaviour (express a preference) only when it does not affect dry matter intake or the proportion of clover in their diet. We conclude that N is not the reason why animals prefer white clover. Manipulating the N concentration in grass will not cause the switch in preference required for animals to easily satisfy their preference from typical mixed species pastures that are grass-dominant and have a low proportion of clover.

Keywords: cattle, diet selection, food preference, grazing behaviour, nitrogen, sheep

Introduction
The proportion of white clover (Trifolium repens L) in mixed species permanent pasture is typically low (e.g. Hoglund et al. 1979). This makes it difficult for sheep and cattle to get a high intake of clover and a high proportion of it in their diet, even by grazing selectively. Furthermore, selective grazing may serve to keep the clover at a low proportion in the sward. Improvements to white clover yield through breeding (1-2% per annum, Woodfield 1999) often do not translate to a higher proportion in the sward, and improvements to grass vigour and persistence impose even greater competitive pressure on clover (Eerens et al. 2001). Creating and maintaining pastures with a high proportion of clover would allow animals to get a high proportion of clover in their diet, and improve animal nutrition and productivity (Clark & Harris 1996), in addition to better capturing the other benefits that white clover offers, such as the fixation of atmospheric nitrogen (N).

Sheep and cattle prefer a diet composed of approximately 70% clover and 30% ryegrass (Lolium perenne L) when they have the opportunity to freely choose (Parsons et al. 1994). This proportion of clover is far greater than can be sustained in mixed pastures. There are many differences between ryegrass and white clover that might explain why animals prefer clover. One of the main nutritional differences is in the concentration of N. Clover usually contains 4-6% N in its leaves, whereas the concentration in grass is variable, often only 2-3% unless receiving fertiliser N, and itself dependent on the transfer of N fixed by clover. Animals fed indoors select a diet that meets their N (protein) requirements for fast growth while avoiding excessive protein intake (Hou et al. 1991; Kyriazakis & Oldham 1993). While the foraging environment at pasture is more complex than for indoor-feeding, there is evidence that animals make food choices on the basis of protein or energy imbalance (Scott & Provenza 2000).

The objective of this study was to determine if the N concentration in grass affects the preference of sheep and cattle for white clover under circumstances where they can freely choose. If N concentration is important in preference between grass and clover we expected that animals would show a lower preference for clover (greater preference for grass) when the grass had a high concentration of N than when it had a low concentration. A reduction in the strong preference for clover would indicate that animals were willing to substitute N-fertilised ryegrass for white clover. As an immediate benefit this would make it easier to satisfy the animals preferred diet composition and nutrient requirements, using mixed pastures with a lower and more easily achieved proportion of white clover. In the longer term identifying the factors that cause preference would enable us to influence that preference, for example by altering the relative distribution of the causative factor (in this case a nutrient) across different species. A reduction in the preference for clover might, paradoxically, result in a higher proportion in the sward and in the diet (Parsons et al. 1991).

Methods
Two experiments were conducted in Manawatu using
established swards of perennial ryegrass (‘Yatsyn’) and white clover (‘Grasslands Pitau’) growing separately, side-by-side. The experiment with dairy heifers was conducted at the Flock House Research area in December 1996, and the experiment using sheep was conducted at the Aorangi Research area in March 2000, on fertile alluvial Rangitikei and Kairanga silt loams at each site, respectively.

Treatments and experimental design
For each experiment, there were three treatments, comprised of choices between ryegrass with a high or a low concentration of N, between ryegrass with high N and white clover, and between ryegrass with low N and white clover, growing separately, side-by-side. Animals could graze freely across both food alternatives. The low N ryegrass received no N fertiliser. Ryegrass with the high concentration of N (similar to white clover) was created by applying urea at 100 and 50 kg N/ha preceding and during the experimental period, respectively. The experiment with sheep used four replications (two spatial replicates, and two temporal replicates created by repeating the experimental measurement sequence with a new group of sheep). For the experiment with cattle there were four spatial replicates of the choice between high and low N grass, and two replicates of each grass-clover choice, laid out in a randomised complete block design.

Sward preparation and management
Treatment plots were mowed prior to animals being introduced to ensure that each species was at a similar sward surface height that was non-limiting to intake. The mean sward surface height was 12 cm for heifers, and 7 cm for ewes.

Animals
Twelve, yearling Friesian dairy heifers (300 ± 12 kg; mean liveweight ± standard deviation) and 12, 30-month old Romney ewes (67 ± 5.5 kg) were each divided into groups of three animals and randomly allocated to treatments. Each group of three heifers was allocated to a plot (0.5 ha, comprised of 0.25 ha of each food item) for 12 days, and the three sheep were allocated to plots (0.22 ha for food choices and 0.11 ha for each food offered alone) for two days. Plots were sufficiently large to ensure that depletion of herbage mass of either species did not affect preference or daily dry matter intake.

Measurements
Grazing time and preference
The primary measurement of the animal response was the total time spent grazing for each treatment, and the time spent grazing on each species. Animals were observed from dawn to dusk, comprising approximately 16 hours/day for cattle and 13 hours/day for sheep. Heifers were observed at 10-minute intervals over six days and their activity (grazing or not grazing) and the species they were on was recorded. Ewes were observed continuously over two days and their predominant activity and species recorded at one-minute intervals. These data were aggregated to derive the mean total time spent grazing each day. Preference was calculated as the proportion of time spent grazing on each food item in a choice.

Grass and clover composition
Samples of ryegrass and white clover were taken by hand-plucking to simulate the herbage being grazed by animals. These samples were frozen and later freeze-dried and ground to pass a 1 mm sieve, and analysed for total N concentration.

Data analysis
The experimental unit was the mean daily time spent grazing for the group of ewes or heifers for the period of observation. Preference was tested against the null expectations of indifference (50:50), and absolute preference for clover (100:0), using t-tests. Grazing times were compared by analysis of variance.

Results
Nitrogen concentration in grass and clover
For the experiment using cattle, the high N grass had 34 g N/kg DM and the low N grass had 18 g N/kg DM (P<0.01), when they were offered as a choice. When each was offered with clover, the N concentration of the high N grass was similar to clover (39 and 47 g N/kg DM, respectively, P>0.05) and the low N grass (25 g N/kg DM) was lower than clover (P<0.01). For the experiment conducted with sheep the concentration of N in the high N grass (45 g/kg DM) was significantly higher (P<0.01) than in the low N grass (32 g/kg DM), and was similar to the concentration in clover (46 g/kg DM).

Grazing time and preference
Cattle
Cattle grazed for a similar total time on the high N grass-clover and the low N grass-clover treatments (520 mins/day and 500 mins/day, respectively, Table 1). This was comprised of 380 mins/day grazing clover and an average of 130 mins/day on grass, indicating a preference for clover, significantly different from 50:50 and 100:0 (P=0.06). Those offered the choice between high N grass and low N grass grazed for 465 mins/day, comprised of 340 mins/day grazing high N grass and 125 mins/day...
grazing low N grass, indicating a preference (P<0.01) for grass with a high concentration of N.

**Sheep**

The total time spent grazing and the time spent grazing on clover and on grass for the high N grass-clover treatment and for the low N grass-clover treatment was not significantly affected by the N concentration in the grass (Table 1), although there was a preference for clover in both treatments (P<0.01). However, sheep offered a choice between grass with a high or a low concentration of N preferred (P<0.05) the high N grass (320 mins/day) compared to low N grass (90 mins/day). When each grass type was offered alone, and there was no choice as to what to eat, the total time spent grazing was similar for each.

**Discussion**

To test the hypothesis that sheep and cattle prefer clover because it has a higher concentration of N, we offered animals different opportunity to respond to N and to express preference, in a structured way. Two of the three different experimental conditions involved only grass as the food items, so as to eliminate the confounding effect between N concentration and the many other traits that differ between ryegrass and white clover. When there was no choice as to what to eat (tested only with sheep), the difference in the concentration of N did not affect the time spent grazing, and by inference the daily dry matter intake. Their only opportunity to regulate the intake of N was by altering dry matter intake. The relatively low nutrient demand for maintenance of these sheep (1200 g DM/day, 19 g N/day, NRC, 1985) and the comparatively high concentration of N even in the grass with low N (33 g N/kg) meant that sheep had little difficulty in satisfying minimum requirements for N intake, and it appears their primary goal on each food type was to meet a target of daily dry matter intake, as indicated by the similar time spent grazing by groups on each food item. It is possible that the higher nutrients requirements of growing cattle (6800 g DM/day, 106 g N/day, NRC, 1984) may have stimulated them to graze for longer each day to compensate for the lower N concentration, if they had been offered this food alone.

When sheep and cattle were offered a choice of grass with a low or a high concentration of N, they preferred that with the high concentration of N. This response indicates that they detected the difference in N concentration of two otherwise similar food items, and expressed a preference, even though in this case, they could easily satisfy their daily dry matter intake requirement from one or other of the food items. Based on the apparent indifference of sheep to the concentration of N when there was no choice, their preference for grass with a high concentration of N is unlikely to be motivated solely by their need for N.

When offered the choice between ryegrass and clover, the concentration of N in the grass did not affect the preference of sheep or cattle for clover. Our hypothesis was that if N were an important nutrient in preference and selection, they would reduce their preference for clover and eat more grass when it had a higher concentration of N. However, this was not the case, and the preference for clover was not significantly affected by the N concentration in the grass (Table 1). Therefore, it appears that the preference for clover is not driven by its N content, but rather by other factors such as its texture, palatability, or digestibility.

**Table 1** Total time spent grazing (mins/day) and preference (ratio of foods eaten) by sheep and cattle offered choices from two food types comprised of ryegrass having nitrogen (N) at a high or a low concentration (HNG_LNG), and each type of ryegrass with white clover (HNG_C, LNG_C), and time spent grazing by sheep offered ryegrass alone, having a high or a low concentration of N (HNG, LNG).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>Food A</th>
<th>Food B</th>
<th>Preference</th>
<th>Significance of difference from:</th>
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<tr>
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<td></td>
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<td>50:50</td>
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<td>100:0</td>
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<td>Cattle</td>
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<tr>
<td>HNG_LNG</td>
<td>465</td>
<td>340</td>
<td>125</td>
<td>73:27</td>
<td>P&lt;0.01</td>
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<tr>
<td>HNG_C</td>
<td>520</td>
<td>380</td>
<td>140</td>
<td>73:27</td>
<td>P=0.06</td>
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<tr>
<td>LNG_C</td>
<td>500</td>
<td>380</td>
<td>120</td>
<td>76:24</td>
<td>P=0.06</td>
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<td>Signif.</td>
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<td>Sheep</td>
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<tr>
<td>HNG alone</td>
<td>360</td>
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<td>LNG alone</td>
<td>350</td>
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<td>Signif.</td>
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<tr>
<td>HNG_LNG</td>
<td>410</td>
<td>320</td>
<td>90</td>
<td>78:22</td>
<td>P&lt;0.05</td>
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<tr>
<td>HNG_C</td>
<td>290</td>
<td>220</td>
<td>70</td>
<td>76:24</td>
<td>P&lt;0.01</td>
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<tr>
<td>LNG_C</td>
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<tr>
<td>Signif.</td>
<td>P&lt;0.01</td>
<td>P&lt;0.05</td>
<td>NS</td>
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</table>

1 For each treatment read Food A and Food B as follows: HNG_LNG: Food A = high N grass, Food B = low N grass; HNG_C: Food A = clover, Food B = High N grass; LNG_C: Food A = clover, Food B = Low N grass.

2 NS = treatment means within columns not significantly different; significant differences among means, and t-tests of preference within treatment indicated by P value.
indications are that this method of forage presentation experimentally in this work, is one strategy to easily required to improve the proportion of clover in mixed fulfil the role of white clover. Management strategies are increases the concentration of N in the grass, does not clover is low, application of fertiliser N, while it increases the concentration of N in the grass, does not fulﬁl the role of white clover. Management strategies are required to improve the proportion of clover in mixed pastures. Growing grass and clover separately, as used experimentally in this work, is one strategy to easily create and sustain a high proportion of clover in both the pasture offered to grazing animals and in their diet. Early indications are that this method of forage presentation beneﬁts animal productivity (Marotti et al. 2001), and might be a simple way to better align forage supply with animal needs.

Conclusions
Sheep and cattle prefer grass with a high concentration of N to that with a low concentration, consistent with observations e.g., of grazing preferentially around urine patches. However, this preference is weakly defended and when offered only a single food item that was low in N, sheep (with a low nutrient demand) made no attempt to compensate for the low concentration by eating more. By contrast, the strong preference of sheep and cattle for clover was defended, but for reasons other than the N concentration of the grass. We conclude that N fertilised grass is not a substitute for white clover for animals. Even when they could satisfy their N (protein) requirements from the fertilised grass, they still preferred a high proportion of clover in their diet. A higher proportion of white clover in pastures is needed to enable grazing animals to achieve their preferred diet composition and realise the high nutritive value of clover for liveweight gain, or milk production. Our inability to sustain a high proportion of clover in swards and in animal diets highlights an urgent need for management strategies to overcome these limitations ahead of more complex changes to nutrients (e.g. carbohydrates) and secondary compounds (e.g. tannins) of clover. The proposed beneﬁts of those manipulations cannot be fully realised when clover forms only a low proportion in the diet. These results do not question the role for strategic use of N fertiliser in grass-dominant pastures to promote pasture production, or for supplementing the N ﬁxed by white clover, although a higher proportion of clover would partially offset those needs.

REFERENCES
