Urine patches indicate yield potential of cocksfoot

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Abstract
Dry matter (DM) production and crude protein (CP%) of sheep urine patches in a grazed cocksfoot pasture (28 day rotation with 21±1 days regrowth) were measured at Lincoln University from September 1999 to May 2001. Also, the DM response from artificial urine patches was measured over time. The rate of nitrogen (N) applied per hectare in an individual urine patch was 173 kg N/ha in autumn compared with 448 kg N/ha in spring. This stimulated a maximum difference in DM production between urine patches and controls over a 21 day period of 1970 kg DM/ha in spring (October). Smaller differences were measured in summer (380 kg DM/ha) and winter (370 kg DM/ha). The maximum difference in CP% was also in spring being 27.0% for urine patches and 18.7% for controls. DM production differences were attributed to differences in net leaf photosynthetic rate, which ranged from 23.5 in spring to 4.5 μmol CO₂ m⁻² s⁻¹ in summer. Leaf photosynthetic rate was limited solely by N supply in spring, by soil moisture (mean soil volumetric water content in the top 500 mm < 20%) and leaf N content (< 3 %N) in summer and mainly by low temperatures (< 9 ºC) but also N supply in winter. The low temperature in winter also meant the duration of urine patch response was 133 days compared with 105 days in spring and 77 days in summer. In a second experiment, the DM response of cocksfoot to synthetic urine (300 kg N/ha) and irrigation was examined in ungrazed areas (four 60-day regrowth periods and one 110-day winter regrowth). From these treatments, the maximum annual DM production for cocksfoot with irrigation and N was 28.6 t DM/ha/yr compared with 9.2 t DM/ha/yr for the control. The addition of irrigation alone yielded 13.0 t DM/ha/yr compared with N alone at 23.5 t DM/ha/yr. To overcome the N stress in cocksfoot pastures a combination of applied N in spring, tap rooted perennial legumes in summer and annual clowers in winter is recommended.

Keywords: clover, cocksfoot, crude protein, Dactylis glomerata, leaf photosynthesis, nitrogen, pasture production, potential yield, urine patches

Introduction
Cocksfoot is a persistent perennial grass species used extensively in dryland farming systems. As for all grasses, its total annual dry matter (DM) production is constrained by a combination of environmental and management factors, including cool season temperatures, summer water deficits, available nitrogen (N) supply and grazing interval. Of these, nitrogen supply is the easiest to manipulate on-farm and offers the greatest potential for changing the pattern of feed supply in dryland environments. The strategic use of nitrogen on cocksfoot pastures has received much less research attention than on ryegrass. However, the lack of clover and presence of obvious, more productive dark green urine patches in cocksfoot pastures during all seasons suggests persistent N stress (Peri et al. 2001). A comparison of urine and non-urine patches throughout the year can be used to indicate the potential yield loss through inadequate N supply. The advantage of this approach is that it integrates the effects of seasonal changes in temperature and water stress and allows the nitrogen response to be isolated. Joshi et al. (1999) indicated that relief of N stress may approximately double pasture production. Furthermore, an increase in grazing preference for cocksfoot from higher herbage N content is likely (Edwards et al. 1993).

The DM response to urine patches has been reported to last for 2-3 months on ryegrass in the more humid Waikato environment (Ledgard et al. 1982) but no similar data is available for cocksfoot.

Thus, the first objective of this study was to quantify the duration of DM and quality (crude protein) responses of cocksfoot pastures to sheep urine patches. The second objective was to ensure pasture was never nitrogen stressed so that the annual DM yield-loss resulting from nitrogen deficits throughout the year could be estimated.

Finally, the third objective was to explain DM responses using a leaf photosynthesis model that responds to environmental inputs.

Materials and methods
Site
The experiment was located at Lincoln, Canterbury, New Zealand (latitude 43º 38´S, longitude 172º 30´E) and was established in September 1990 as an unshaded pasture for comparison with pastures under trees in a silvopastoral experiment. For the current experiment only the four unshaded ‘Grasslands Wana’ cocksfoot plus ‘Grasslands Huia’ white clover main plots (27.5 x 18.0 m) were measured from September 1999 to May 2001. Clover content of the pasture was low (< 10%) in all seasons. The soil is classified as a Templeton silt loam (Karageorgis et al. 1984). The average annual evaporation
is about double the mean rainfall of 660 mm. No fertilizer, lime or irrigation have been applied to the experimental area since its establishment. Soil tests in September 1999 indicated low soil fertility (pH 5.9, Olsen P 7.5 μg/ml, K 0.36 m.e./100g, S(SO₄) 3.5 ppm). A flock of Coopworth ewe lambs were rotationally grazed for 7±1 days around the four cocksfoot main plots giving a 28 day rotation with 21±1 days regrowth.

**Urine patches**

Two days after each grazing finished, 10 easily identifiable new sheep urine patches per replicate were identified in the main cocksfoot plots. At the same time paired control areas, adjacent to urine patches, were also selected from within 1 m of each selected urine patch. This gave a total of 20 sampling points per replicate. The duration of urine patch response was estimated as the time from identification of the patch until no significant difference in DM was measured between urine and non-urine patches. These data were analysed as a completely randomised block design.

**Synthetic urine x irrigation experiment**

A second experiment was established in September 1999. This was designed to examine the maximum productivity and main yield changes in cocksfoot due to N and irrigation during all seasons. This experiment was conducted in a series of fenced 6.6 x 6.0 m areas within the main plots. Irrigation (0 or fully) and nitrogen (0 or 300 kg N/ha) were applied to 2.47 m² areas as a completely randomised 2x2 factorial design with two replicates.

The four treatments were monitored for four 60-day regrowth periods (1 September - 30 October 1999; 1 November - 30 December 1999; 6 January - 6 March 2000; and 8 March – 7 May 2000). A further 110-day winter regrowth period was measured from 8 May - 16 August 2000. After each period (60 or 110 days), the next 6.6 x 6.0 m area was fenced in a new position in the grazed pastures of the main plot and each treatment was re-imposed. The full irrigation treatment was timed to prevent the soil moisture deficit from exceeding 35 mm, or a reduction in volumetric water content (VWC) of 7% in the top 500 mm. Over the two seasons a mean of 185 and 287 mm of water were applied at an average rate of 15-22 mm per application for non-N and N treatments, respectively. At the start of each regrowth period a single application of N as synthetic sheep urine (Fraser *et al*. 1994) was made.

**Environmental and herbage measurements**

Air temperature was recorded with a digital temperature

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**Figure 1** Mean soil volumetric water content (VWC) (%) at 0-500 mm soil depth (—•—) and mean daily air temperature (°C) (—) at Lincoln University (Canterbury). Bars indicate standard error of the mean (SEM) for soil VWC.
sensor. The seasonal changes in mean daily temperature during the period of this experiment are shown in Figure 1. The mean soil moisture content in the top 500 mm was measured every 10 days with Time Domain Reflectometry. In spring, soil moisture was always above 24% and was always more than half the maximum available water content of the site (mean field capacity = 30%) indicating that the treatments were not moisture stressed at that time (Figure 1). However, in summer and autumn 2001, pastures were under water stress as indicated by soil VWC lower than 20%.

Pasture samples for DM production were obtained from a 0.2 m² quadrat cut to 20-25 mm stubble height. A small circular quadrat (diameter 250 mm, 0.05 m²) was used to sample individual ± urine patches (mean diameter ranged from 200 to 300 mm). DM samples were dried in a forced draft oven at 65 ºC to constant weight. The herbage N content from each DM cut was determined using the Kjeldahl technique.

The area which urine patches covered in main plots was measured using six permanent line transects across the cocksfoot pastures in October (spring), January (summer) and April (autumn) of 1999 and 2000. The mean diameter of individual urine patches and the distances between urine patches were measured using a tape placed on transects.

Urine was collected from sheep grazing the cocksfoot pastures to establish the amount of nitrogen applied in urine patches. Urine samples were taken in autumn (18 April 2000) and spring (24 October 2000) from 5 animals and total nitrogen determined using the Kjeldahl technique.

Leaf photosynthesis model
To evaluate the integrated effect of N, temperature and water stress on DM production in urine patches over time, the multiplicative model for cocksfoot leaves described by Peri et al. (2002) was used. The ranges for predicting net leaf photosynthesis are: (i) air temperatures from 2 to 37 ºC, (ii) water status from predawn leaf water potential (φw) –0.1 to –16.0 bar (corresponding to a soil VWC in the top 500 mm of 8.5 to 34%), (iii) foliage N content from 1.5 to 5.9%. To predict leaf photosynthetic rate (Pmax), actual values from field measurements of N content, soil VWC and temperature were used.

Results
DM growth rate of new urine patches
The mean area of the plots covered by visually obvious urine patches varied from 25% in October (1999/2000) to 32% in April (1999/2000) with a mean patch diameter...
of 0.22 m. Sheep urine had a higher N concentration (g/l) in spring (October) than in autumn (April) (Table 1). Results were used to estimate the rate of N applied per hectare based on a mean urination volume of 0.15 l for young sheep (Haynes & Williams 1993) and ranged from 173 in autumn to 448 kg N/ha in spring.

The cocksfoot DM growth rates from individual new urine patches compared with non-urine areas are shown in Figure 2. The seasonal fluctuations showed a mean maximum growth rate of 130 kg DM/ha/d in October-November for urine patches which was three times higher (P< 0.05) than the non-urine areas. These differences decreased in summer and autumn.

**Urine patch dynamics**

**DM production**

The relative difference in DM production between urine and non-urine patches and the duration of the effect of urine patches (when DM was equal to controls) on pasture production varied over seasons (Figure 3). DM production was greater (P< 0.05) than controls at the first rotation (new urine patches), but declined over time. For example, the maximum difference in DM production between urine patches and controls was about 380 kg DM/ha in summer (Figure 3a) and winter (Figure 3b) but up to 1970 kg DM/ha during spring (Figure 3c). The duration of the effect of urine on DM production was estimated at 77 days in summer, 133 days in winter and 105 days in spring.

**Crude protein**

The actual values of CP%, the relative difference in CP% between urine patches and controls and the duration of the effect of urine on pasture CP% also differed over seasons (Figure 4). The CP% was greater (P< 0.05) than controls for the first period (new urine patches) but the difference declined over time. The maximum difference in CP% was recorded in spring as 27.0% for urine and 18.7% for non-urine patches. The duration of the response to urine for CP% was similar to those for DM production (Figure 3).

**Leaf photosynthesis**

The mean values of environmental and herbage variables measured during the growing periods of urine patches and controls are summarised in Table 2. The relative difference in net leaf photosynthesis between urine and non-urine patches, and the duration of the

### Table 1  Nitrogen (N) concentration of sheep urine in autumn (April) and spring (October) 2000, and the estimated rate of N applied from sheep urine per hectare to cocksfoot pastures.

<table>
<thead>
<tr>
<th>Season</th>
<th>N (g/l)</th>
<th>Mean urination volume</th>
<th>N applied per urination</th>
<th>Mean urination area</th>
<th>N in mean urine patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>3.46</td>
<td>0.15</td>
<td>0.52</td>
<td>0.03</td>
<td>173</td>
</tr>
<tr>
<td>Spring</td>
<td>8.97</td>
<td>0.15</td>
<td>1.35</td>
<td>0.03</td>
<td>448</td>
</tr>
</tbody>
</table>

*Mean urination volume from Haynes & Williams (1993).*
Table 2  Mean (Tmean), maximum (Tmax) and minimum (Tmin) daily temperature, mean nitrogen percentage of grass (N%), mean monthly photosynthetic photon flux density (PPFD) and mean volumetric water content (VWC) in the top 500 mm of soil for three growing periods in urine and non-urine patches. Values were used to predict leaf photosynthetic rate of cocksfoot plants at Lincoln University (Canterbury).

<table>
<thead>
<tr>
<th>Season</th>
<th>Date</th>
<th>Tmean (°C)</th>
<th>Tmax (°C)</th>
<th>Tmin (°C)</th>
<th>N urine (%)</th>
<th>N control (%)</th>
<th>PPFD (mol/m²/month)</th>
<th>VWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>2 Jan-27 Feb 00</td>
<td>15.0</td>
<td>20.5</td>
<td>11.5</td>
<td>2.8</td>
<td>2.2</td>
<td>1620</td>
<td>19.2</td>
</tr>
<tr>
<td>Winter</td>
<td>11 Apr-2 Aug 00</td>
<td>8.8</td>
<td>14.1</td>
<td>5.0</td>
<td>4.1</td>
<td>3.0</td>
<td>450</td>
<td>29.0</td>
</tr>
<tr>
<td>Spring</td>
<td>10 Oct 00-2 Jan 01</td>
<td>12.3</td>
<td>18.6</td>
<td>7.8</td>
<td>3.7</td>
<td>2.9</td>
<td>1560</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Note: Mean nitrogen content was calculated based on crude protein data over duration of urine patch from Figure 4.

Figure 4  Response over time of crude protein percentage of cocksfoot from urine (●) and control (○) patches starting in: a) summer (13 December 1999), b) winter (22 March 2000) and c) spring (21 September 2000). Bars indicate standard error of the mean (SEM).

Figure 5  Response over time of predicted leaf photosynthetic rates of cocksfoot urine (●) and control (○) patches starting in: a) summer (13 December 1999), b) winter (22 March 2000) and c) spring (21 September 2000).
effect of urine patches, also differed between seasons. The range was from 23.5 in October to 4.5 μmol CO₂ m⁻² s⁻¹ in February 2000 (Figure 5). The maximum difference in net leaf photosynthesis between urine patches and controls was greater (P<0.05) after the first 21 day rotation (new urine patches). Specifically, Pₘₐₓ values were 17.5 vs 13.4 μmol CO₂ m⁻² s⁻¹ in summer (Figure 5a), 18.1 vs 14.4 μmol CO₂ m⁻² s⁻¹ in winter (Figure 5b) and 23.5 vs 19.0 μmol CO₂ m⁻² s⁻¹ in spring (Figure 5c), for urine vs control patches, but differences declined over time. The duration of the response to urine on leaf photosynthesis was the same as that found for DM production in each season.

### Discussion

The maximum potential DM production was 28.6 t/ha/yr for this sub-humid temperate environment. This was achieved from exclosure plots that were fully irrigated, received 300 kg N/ha as synthetic urine in each of five regrowth periods and were allowed to regrow for 60-110 days after grazing. This result is similar to yields reported in the Netherlands for perennial ryegrass (28 t/ha) when 125 kg N/ha and 62 kg K/ha were applied in each regrowth period (Alberda & Sibma 1968). To achieve these yields growth rates over 150 kg DM/ha/d (Figure 2) are required which are consistent with those reported by Lemaire et al. (1983) for cocksfoot grown in France.

In practice this yield is unlikely to be achieved. Grazing management would aim to minimize the amount of reproductive material in the pasture, which contributed nearly 2 t/ha, and the rotation length would be shorter than the 60 day exclosures used during the growing season in this study. However, these results do provide insights into the environmental and management constraints operating on annual cocksfoot pasture production.

### Spring response to N

The large response to N in all seasons increased yield from 9.2 to 23.5 t/ha compared with 13.0 t/ha when only water was added (Table 3). The strong N response was particularly apparent during the spring (Figure 2). This highlights the potential production loss from the typical state of N stress. Specifically, the nitrogen fertilised artificial urine patches produced 2 t/ha more than control patches by October 10. This response occurred when soil moisture content and temperature were non-limiting and implies that the cocksfoot pastures were severely

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Regrowth period (kg DM/ha)</th>
<th>Total annual (t DM/ha/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sep-Oct 99</td>
<td>Nov-Dec 99</td>
</tr>
<tr>
<td>Control</td>
<td>2650</td>
<td>3260</td>
</tr>
<tr>
<td>Irrigated</td>
<td>2650#</td>
<td>5340</td>
</tr>
<tr>
<td>N</td>
<td>5380</td>
<td>7620</td>
</tr>
<tr>
<td>Irrigated + N</td>
<td>5380#</td>
<td>8970</td>
</tr>
<tr>
<td>SEM</td>
<td>110.1</td>
<td>98.7</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irrigation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Irrigation x N</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Irrigation was not required, therefore values are the same as non-irrigated treatments.

P<0.05; ** P<0.01; *** P<0.001; ns= no significant differences.
nitrogen deficient during this time. The leaf photosynthesis results highlight this limitation with urine patch areas operating at near maximum (Peri et al. 2002) values and control plots approximately 20% lower throughout the spring/early summer period (Figure 5c). Simply, this indicates a 20% reduction in potential photosynthesis per leaf per day that accumulates to cause the observed reduction in DM yield.

The crude protein results support the assumption that N stress has a large negative effect on spring production. The urine patches had a maximum value of 27% CP in October, which is equivalent to a leaf N concentration of 4.4%, compared with only 18% CP or 2.9% leaf N in the controls (Figure 4c).

The direct result of these differences in leaf N concentration would be a 20% lower leaf photosynthesis rate as estimated for the control plots compared with the urine patches (Figure 5c). The difference between patches decreased later in the spring as the extra dry matter production in the urine patches (Figure 2) diluted the additional available N, so that by 2nd January the CP values were both about 20% (Figure 4c). Thus, despite the high N concentration (448 kg N/ha) in the urine during spring time (Table 1) the leaf photosynthesis in urine and non-urine patch areas would be severely restricted by the leaf N concentration of 2.4% (Figure 5c). A secondary effect of the low leaf N is likely to be a reduction in grazing preference during this period (Edwards et al. 1993), except for in any new urine patches.

Summer response to N
As expected, during summer both N and moisture stresses were observed. The DM production of urine patches was only 38% higher than controls in the first January rotation (Figure 3a). This smaller response reflects the combination of low leaf N (2.8%) and a mean soil VWC of less than 20% (Figure 1). The leaf photosynthesis rates during this period reflect the 25% increase from the urine patches but this value was only 70% of the maximum and declined to be similar to the non-urine rate (6 μmol CO2 m-2 s-1) in February (Figure 5a) as water stress became more severe (Figure 1).

The implication was that during periods when the top soil was dry the added urine N was largely unavailable to the plants. This is consistent with a restriction in the movement of nitrate and ammonium ions from the urine patches to the plant during such periods, even when water is available from depth in the profile (Lemaire & Denoix 1987). Similarly, Garwood & Williams (1967) reported that during periods of drought, which dry out the soil surface (150-200 mm), there is reduced N uptake and mineralisation of organic N.

During this summer period the increase in DM production from urine patches lasted about 2 months (Figure 3a) which was consistent with previous studies (During & McNaught 1961; Ledgard et al. 1982). The continued water stress meant grazing had to be discontinued in late February and March of both seasons (Figure 1). Thus, any possible relief from applied nitrogen during this period would be of limited effectiveness because of severe soil moisture deficit.

Winter response to N
By winter, the N content in non-urine areas was still deficient and mean air temperatures were sub optimal at <9°C (Figure 1) but soil moisture levels were non-limiting (Table 2). As a consequence leaf photosynthesis was restricted mainly by temperature during this period (Figure 5b). It follows that N demand would be low with the slow growth (Figure 2) which is consistent with the extended duration of response (133 days) for the urine patches.

Despite the restrictions on photosynthesis and growth by low temperature the crude protein of urine patches averaged about 25% through the autumn and winter compared with the increase from 14 to 23% observed for the controls from April to July (Figure 4b).

Management of N stress in cocksfoot pastures
The overall differences in seasonal responses of cocksfoot DM production highlight the importance of understanding the environmental and management constraints to develop an effective strategy to maximise production. The results of this study highlight low N status of cocksfoot in non-urine patches throughout the year. This has been shown to limit pasture production by restricting photosynthesis rates but is also likely to influence grazing preference of cocksfoot based pastures. Thus, a three pronged N management strategy is proposed. Firstly, the application of artificial N in the spring, when moisture is non-limiting and temperature rising is expected to produce the greatest DM response per unit of N applied. However, further applications of N fertiliser in summer and autumn appear unjustified. This is because without water the N would not be utilised in summer and low temperatures will restrict winter growth. The introduction of tap-rooted legumes, such as Caucasian clover to access moisture below the cocksfoot rooting zone may provide an opportunity for improving the leaf N concentration and palatability of pastures in summer. Similarly, the greater winter activity of annual clovers may provide a source of nitrogen to maintain leaf N levels when temperatures are low in late autumn and winter.

Conclusions
1) Cocksfoot pasture production was limited by
nitrogen supply, which affects leaf photosynthesis rates, in all seasons.
2) The magnitude of DM response to applied N was greatest in the spring but restricted by moisture stress in summer and low temperatures in winter.
3) It is proposed that the productivity and the palatability of cocksfoot may increase by relieving N stress through fertiliser application in spring and the inclusion of tap rooted perennial and annual clovers may enhance summer and autumn/winter production, respectively.

REFERENCES