A review of unpublished New Zealand trials examining diurnal variation and diet effects on faecal and urinary nitrogen concentrations in dairy cattle

Gibbs, S. J., Muir, P. D., Thomson, B. C., Bown, M.D.

May 2014

Introduction

Farmed animals in New Zealand contribute around 46% of the country's greenhouse gas output. Of this, approximately 69% is methane (CH_4) and 31% is nitrous oxide (N_2O), with 80% of the latter resulting from nitrification and denitrification of urinary nitrogen by soil microorganisms and, to a much lesser extent, faecal nitrogen. To date, models evaluating the nitrogen (N) excretion of ruminants have assumed that N excreted in urine is constant and research on mitigating N_2O output has largely focussed on nitrification inhibitors such as dicyandiamide (DCD), stand-off pads to keep stock off pastures in wet conditions and feeding low N feeds such as maize silage.

However, there is some evidence from dairy cows that urinary nitrogen concentration is correlated with diurnal grazing behaviour (Betteridge et al. 2013; Clark et al. 2010). Therefore, a strategy as simple as changing the time of feeding (i.e. time of shifting onto a new feed break) might offer potential reductions in N_2O emissions.

This paper uses unpublished results from recent New Zealand experiments to examine potential cyclic variation and diet influences on faecal and urinary nitrogen concentrations in dairy cattle.

Methods

Results from twenty two experiments measuring urinary and faecal nitrogen concentrations from between three and eight dairy cattle at four hourly intervals were combined into a database. Experiments varied in terms of feeding time (morning or afternoon) and feed type (grass, kale or fodder-beet) (Table 1).

Data were examined for patterns in nitrogen excretion relative to feeding time and the effects of diet on these patterns.

Table 1: Experiments contributing to the database

Number of experiments*	Cattle type	Feed type	Time of feeding
6	Dairy breed (lactating cows)	Grass at pasture (ryegrass)	Afternoon: new feed break at 4pm
4	Dairy breed (bulls)	Grass silage (ryegrass)	Morning: 8am
6	Dairy breed (non-lactating cows)	Kale supplemented with cereal straw	Morning: 8am
6	Dairy breed (non-lactating cows)	Fodder beet supplemented with cereal straw	Morning: 8am

Results

EFFECT OF TIME OF FEEDING

Faecal N components

Regardless of feeding time (8am or 4pm) mean faecal NH_3 followed a similar pattern after feeding (Figure 1). Maximum faecal NH_3 concentration occurred at feeding time, thereafter falling to a minimum 12 hours later. However, overall mean concentration was similar between the two feeding times suggesting there was little overall impact on the amount of ammonia produced (Table 2).

Diurnal patterns in faecal NH_3 excretion were very variable between individual experiments, particularly in afternoon fed animals (Figure 2). Individual experiments with lower mean values tended to have a flatter diurnal pattern with lower points closer to feeding.

Table 2.Pattern of mean faecal NH_3 concentration (mmol/l) from dairy cattle fed pasture at two different times. Bold figures correspond with feeding times.

	Time of day						
Feeding time	**8am**	**12pm**	**4pm**	**8pm**	**12am**	**4am**	**Mean**
Grass: 8am	**7.417**	6.340	6.339	5.644	7.016	6.828	6.597
Grass: 4pm	6.559	7.054	**7.590**	6.958	6.099	5.708	6.661

Figure 1. Mean faecal NH_3 concentration (mmol/l) in pen-fed dairy bulls fed grass silage in the morning (8am) and in lactating dairy cows fed grass at pasture in the afternoon (4pm). Arrows correspond with feeding times.

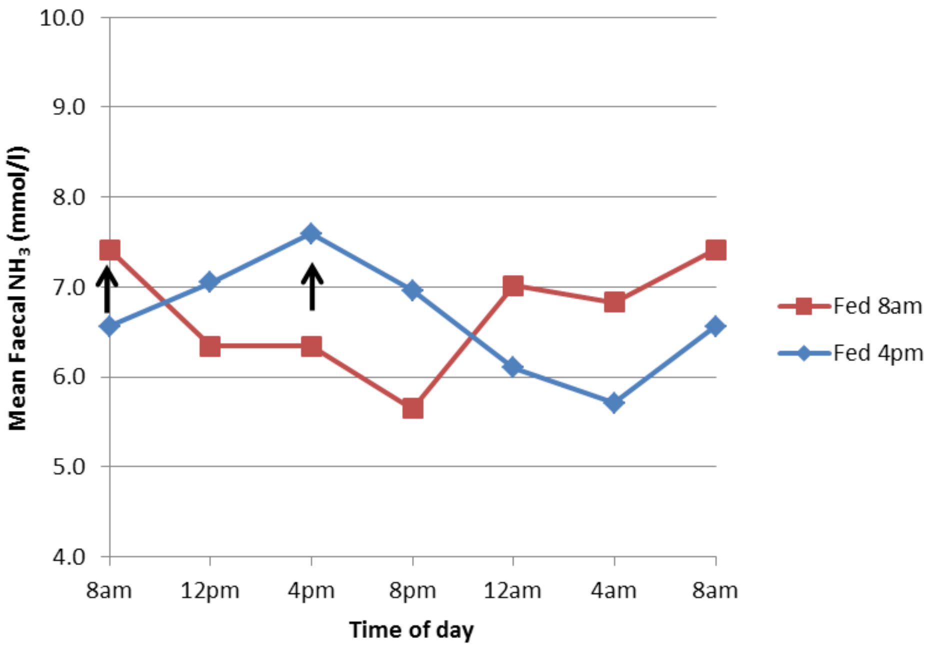
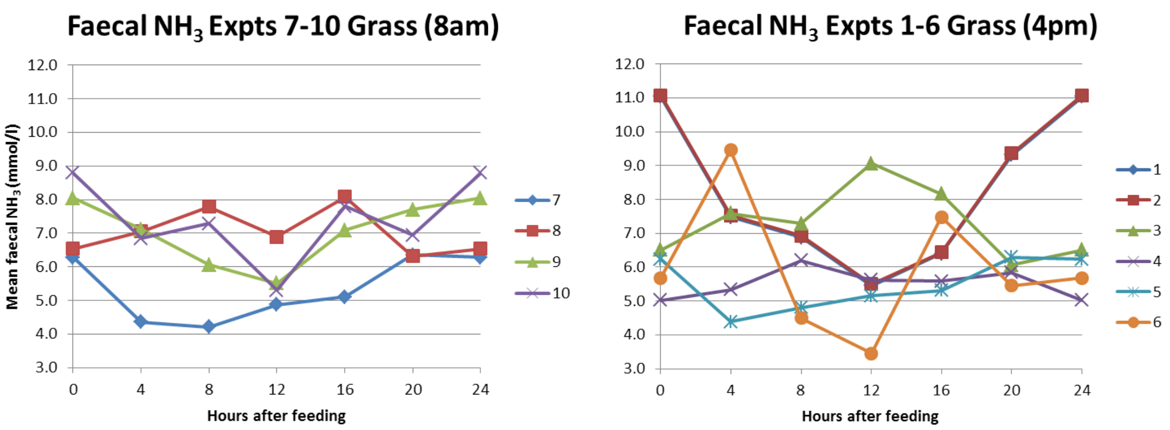


Figure 2. Between-experiment variability in mean faecal NH_3 concentration (mmol/l) from ten individual experiments in cattle fed grass in the morning (8am) or afternoon (4pm).



As for mean faecal NH_3, regardless of feeding time, mean faecal urea concentration followed a broadly similar pattern over the 24 hours after feeding (Figure 3) despite high variability between individual experiments (Figure 4). Maximum faecal urea concentration typically occurred 4 to 8 hours after feeding thereafter falling to a low concentration 12 hours after feeding. However, daily mean faecal urea concentration was greater in the animals fed in the morning (Table 3). This may be due to differences in overall N output or differences in experimental protocols which require further investigation.

However, the pattern of faecal urea excretion was variable between experiments; some experiments showed an apparent cyclical pattern whilst others did not, as well as large differences in the concentrations (Figure 4). As with faecal NH_3 those experiments with the lowest overall urea concentrations had the smallest diurnal variation and peak values occurred earlier. Three of 4 studies fed in the afternoon showed reductions in faecal urea concentration 8-12 hours post feeding with largest reductions occurring in those with the largest urea outputs. Very low faecal urea concentrations in other studies suggest that these may be endogenous losses which are not affected by feeding.

Table 3. Mean faecal urea concentration (mmol/l) from dairy cattle fed pasture at two different times. Bold figures correspond with feeding times.

	Time of day						
Feeding Time	**8am**	**12pm**	**4pm**	**8pm**	**12am**	**4am**	**Mean**
Grass: 8am	**31.781**	35.756	39.921	29.638	35.681	27.725	33.417
Grass: 4pm	23.755	24.960	**25.397**	33.481	27.950	20.076	25.936

Figure 3. Mean faecal urea concentration (mmol/l) in pen-fed dairy bulls fed grass silage in the morning (8am) and in lactating dairy cows fed grass at pasture in the afternoon (4pm). Arrows correspond with feeding times.

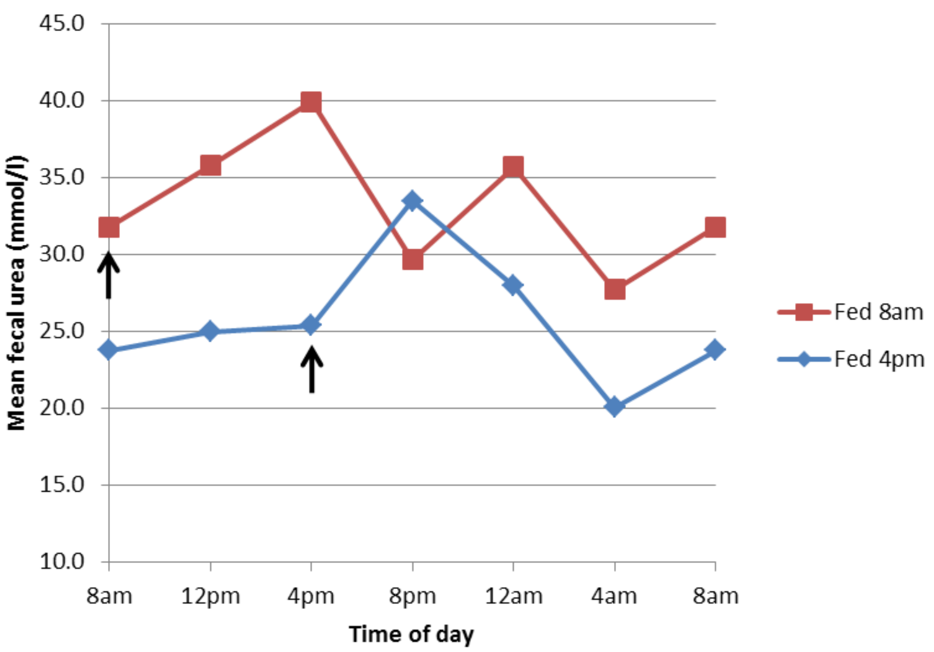
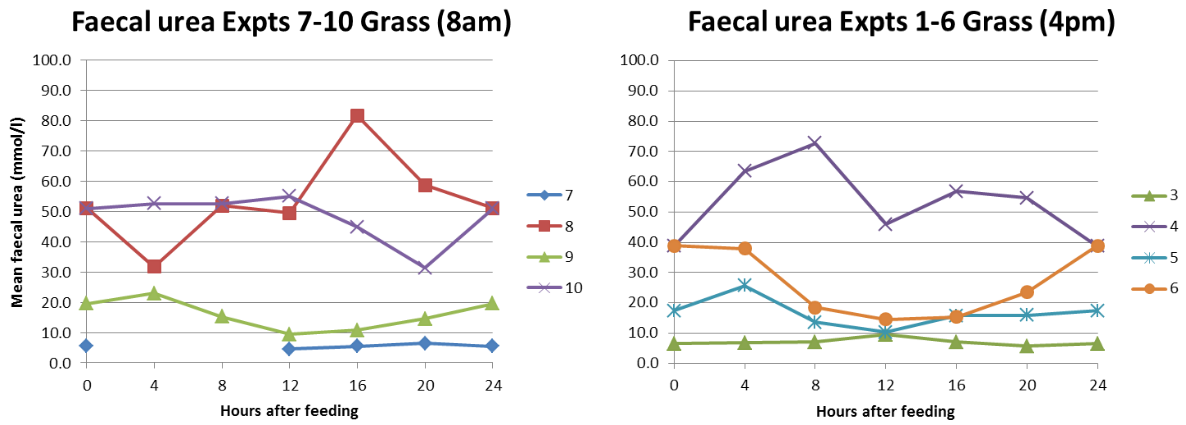


Figure 4. Between-experiment variability in faecal urea concentration (mmol/l) from eight individual experiments in cattle fed grass in the morning (8am) or afternoon (4pm).



EFFECT OF DIET

Faecal N components

Diurnal variation in faecal NH_3 concentration was apparent for grass and kale but less obvious for fodder beet (Figure 5 and 6). For grass, faecal NH_3 appeared to decline to a minimum 12 hours after feeding followed by a return to pre-feeding level by 24 hours. The pattern was, however, very variable between grass-fed experiments (Figure 6). Conversely, faecal NH_3 concentration for kale and fodder beet appears to peak at 12 hours after feeding. This appears to be fairly consistent across individual experiments (Figure 6). In general, across all feeds, experiments with higher overall faecal NH_3 concentration had the largest diurnal variation in faecal NH_3 concentration.

Faecal NH_3 concentration in kale-fed animals was higher than for grass-fed and fodder beet-fed animals throughout the diurnal cycle (Table 5, Figure 5). This was consistent across individual experiments for each feed type (Figure 6) and at all time-points over the 24 hour period (Table 5).

Table 5. Mean faecal NH_3 concentration (mmol/l) in dairy cattle at four hourly intervals from feeding grass, kale or fodder beet.

	Hours after feeding						
Feed type	**0**	**4**	**8**	**12**	**16**	**20**	**Mean**
Grass†	7.417	6.340	6.339	5.644	7.016	6.828	6.597
Kale	11.399	11.675	10.706	16.148	12.064	11.167	12.193
Fodder beet	5.327	5.922	5.547	6.066	5.859	5.600	5.720

† Data from morning and afternoon grass-fed cattle are combined

Figure 5. Mean faecal NH_3 concentration (mmol/l) in dairy cattle fed grass, kale or fodder beet.

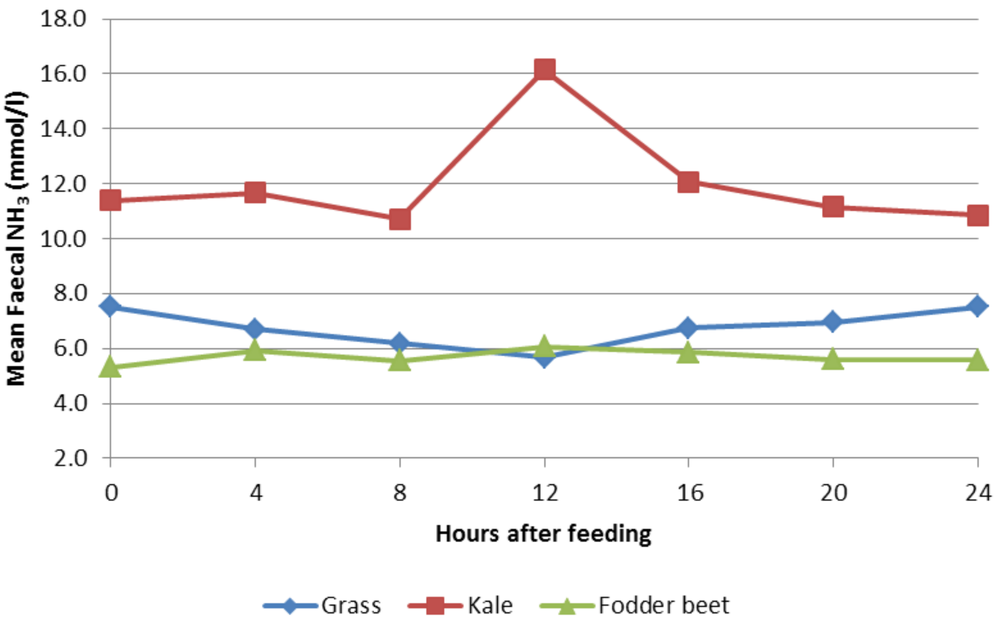
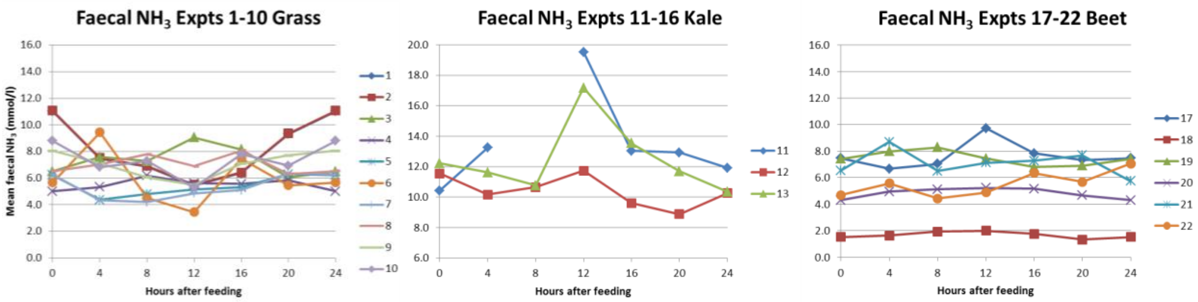


Figure 6. Between-experiment variability in mean faecal NH_3 concentration (mmol/l) from 19 individual experiments with cattle fed grass, kale or fodder beet.



While there did appear to be some diurnal variation in faecal urea concentration in cattle fed either grass or fodder beet (Table 6, Figures 7 and 8), it varied widely between studies. As with faecal NH_3, experiments with high concentrations of faecal urea also showed the greatest diurnal variation. Diurnal patterns for faecal urea appeared to be different for grass and fodder beet, the former characterised by a peak between 4 and 18 hours after feeding, and the latter by a decline to minimum between 4 and 8 hours.

Table 6. Mean faecal urea concentration (mmol/l) in dairy cattle at four hourly intervals from feeding grass or fodder beet.

	Hours after feeding							
Feed type	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**Mean**
Grass†	19.059	24.412	25.290	20.571	25.145	24.228	27.059	23.118
Fodder beet	18.741	13.439	14.821	15.331	17.604	19.447	19.123	16.534

† Data from morning and afternoon grass-fed cattle are combined

Figure 7. Mean faecal urea concentration (mmol/l) in dairy cattle fed grass or fodder beet.

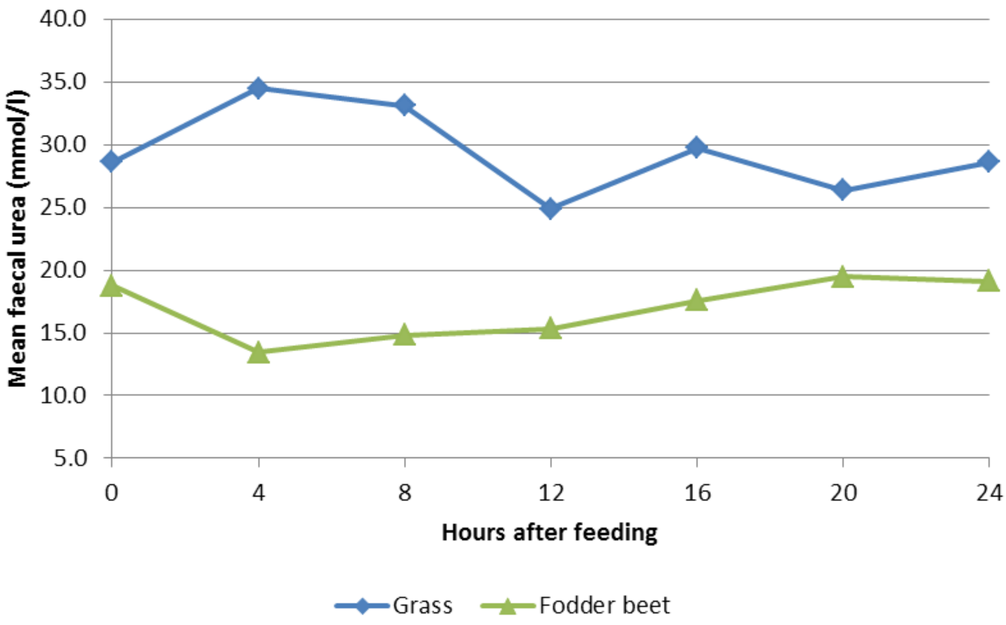
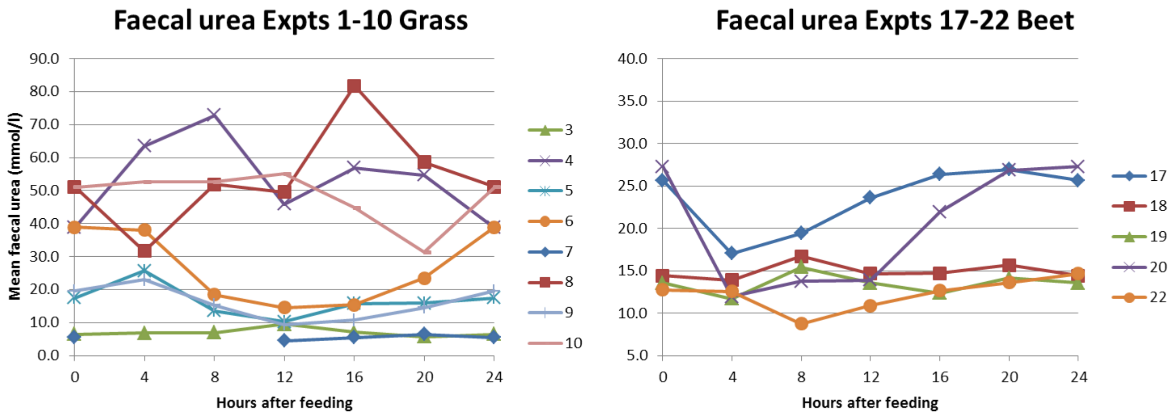


Figure 8. Between-experiment variability in mean faecal urea concentration (mmol/l) from 13 individual experiments with cattle fed grass or fodder beet.



When all nitrogen components are combined, there appeared to be no consistent pattern of diurnal variation in mean faecal N% in animals fed grass or kale. However there appears to be a more consistent diurnal pattern for animals fed fodder beet with a decline to a low point in faecal N% 12 hours after feeding (Figures 9, 10).

Faecal N% appeared to be consistently higher in grass-fed cattle compared to those fed kale and fodder beet. However, there appeared to be little overall difference in faecal N% between animals fed kale or fodder beet (Table 7, Figures 9 and 10).

Table 7. Mean faecal total N% in dairy cattle at four hourly intervals from feeding grass, kale or fodder beet.

	Hours after feeding							
Feed type	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**Mean**
Grass	3.067	3.147	3.054	3.034	3.087	3.180	3.067	3.095
Kale	2.173	2.157	1.996	2.246	2.172	2.122	2.150	2.144
Fodder beet	2.062	2.013	1.933	1.891	1.997	2.059	2.095	1.993

Figure 9. Mean faecal N% at four hourly intervals in dairy cattle fed grass, kale or fodder beet.

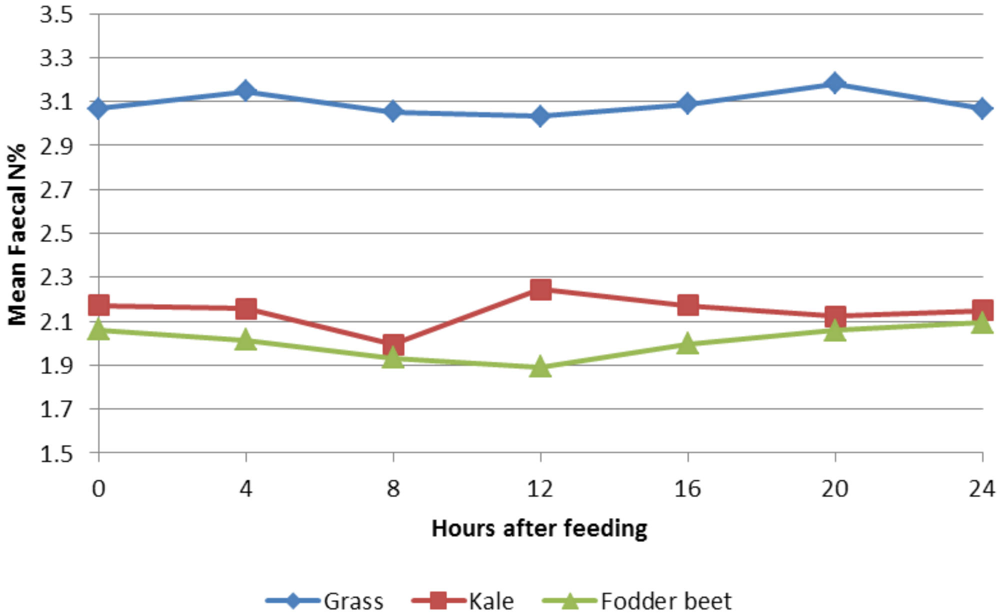
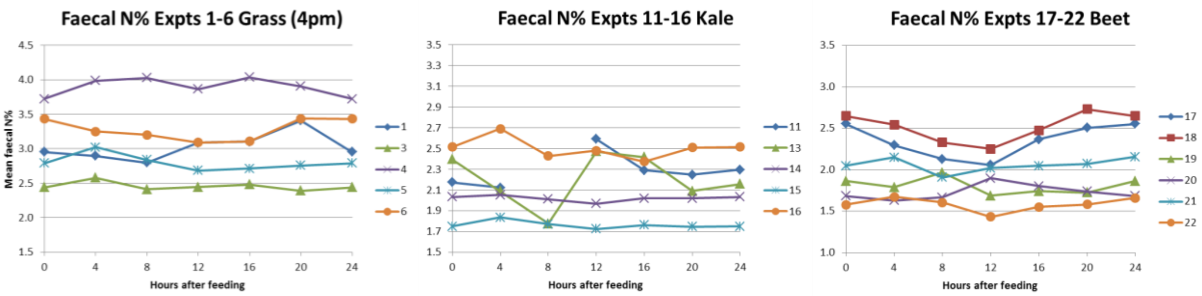


Figure 10. Between-experiment variability in mean faecal N% from 16 individual experiments with cattle fed grass, kale or fodder beet.



Urinary N components

Mean urinary NH_3 concentration appeared to peak between 8 and 16 hours after feeding, across all feed types (Figure 11). However, the pattern was variable between individual experiments. Some experiments showed distinct diurnal variation whist others did not. As with faecal N components, those with the greatest N output had the greatest diurnal variation (Figure 12).

Table 8. Urinary NH_3 (mmol/l) in dairy cattle fed grass (4pm), kale (8am) or fodder beet (8am).

	Hours after feeding							
Feed type	0	4	8	12	16	20	24	Mean
Grass	2.328	4.436	4.313	5.552	4.831	4.296	2.328	4.293
Kale	2.363	2.985	3.335	4.541	2.394	2.115	2.644	2.956
Fodder beet	4.734	3.392	5.184	2.987	1.796	3.270	4.731	3.561

Figure 11. Mean urinary NH_3 concentration (mmo/l) in dairy cattle fed grass, kale or fodder beet.

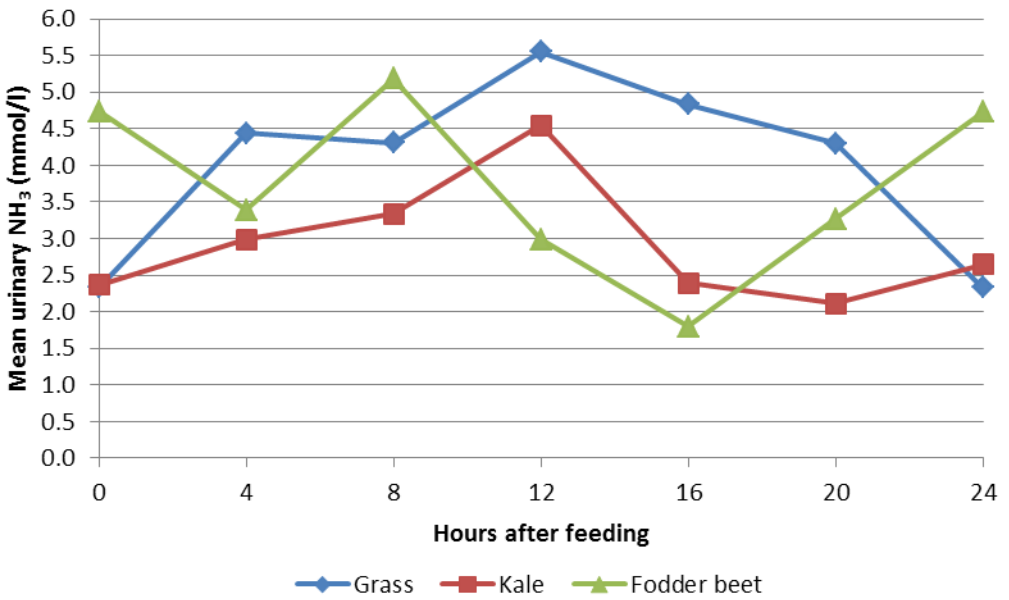
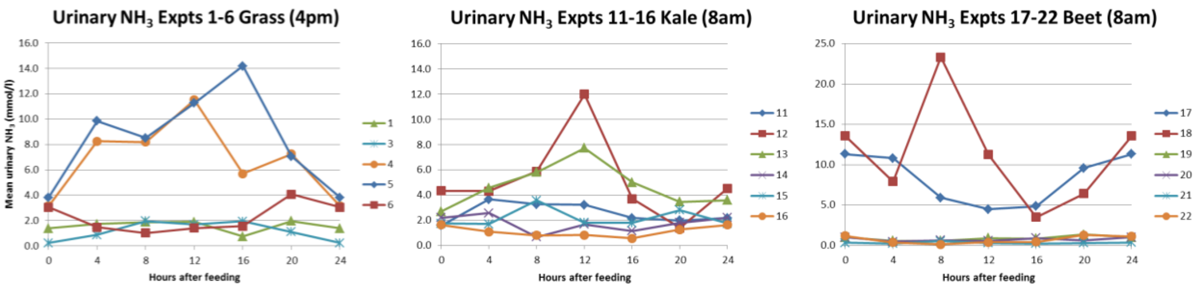


Figure 12. Between-experiment variability in mean urinary NH_3 concentration (mmol/l) from 18 individual experiments with cattle fed grass, kale and fodder beet.



As with faecal urea output, diurnal variation in urinary urea concentration was apparent for all feed types but the pattern was variable and inconsistent between individual experiments on grass and fodder beet. In kale-fed cattle the peak urinary urea concentration was between 8 and 16 hours after feeding (Figure 14). As with other faecal and urinary N components, experiments with higher overall N output had greater diurnal variation.

There was a two- to three-fold difference in overall mean urinary urea concentration between grass-fed and fodder beet-fed animals (Table 9, Figure 13). This was consistent across the individual experiments (Figure 13).

Table 9. Mean urinary urea (mmol/l) at four hourly intervals in dairy cattle fed grass, kale or fodder beet

	Hours after feeding							
Feed type	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**Mean**
Grass	145.64	132.06	119.62	142.14	156.66	147.00	145.64	140.521
Kale	90.05	149.31	166.56	157.94	156.51	112.37	82.97	138.791
Fodder beet	57.74	65.64	60.66	44.66	39.64	48.17	57.17	52.751

Figure 13. Mean urinary urea concentration (mmol/l) at four hourly intervals in dairy cattle fed grass, kale or fodder beet.

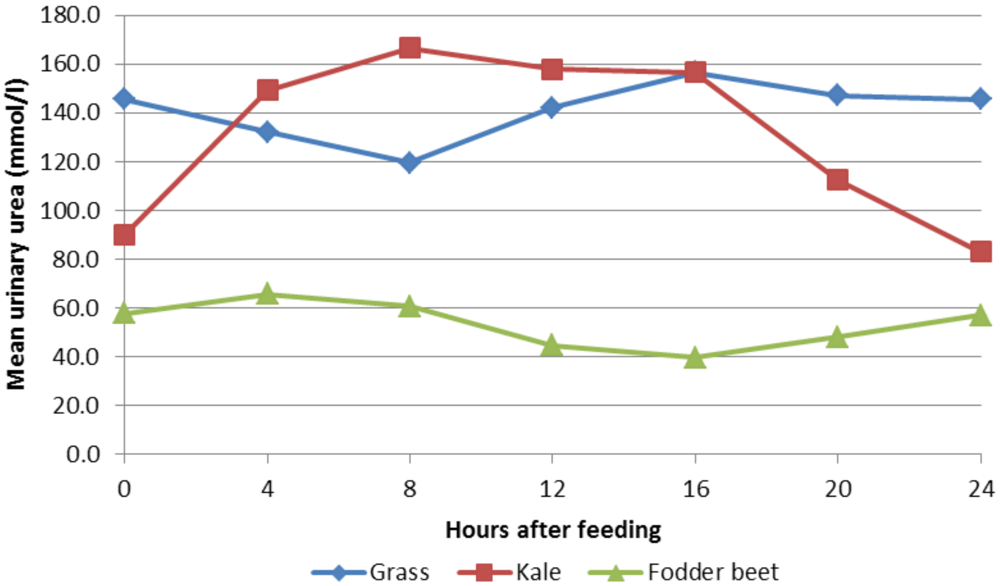
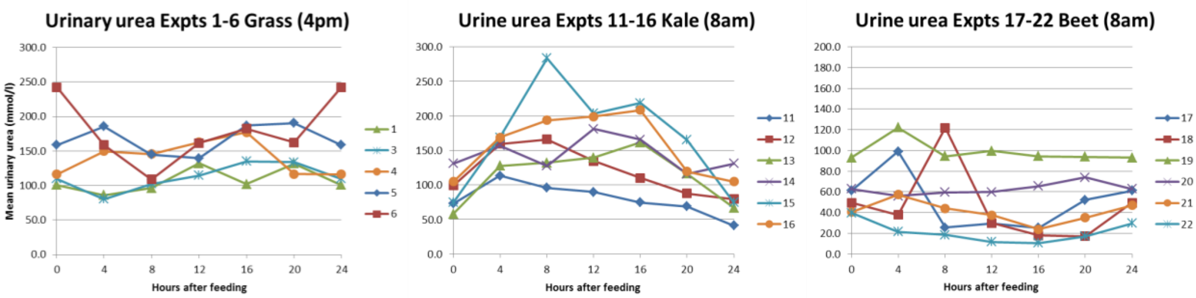


Figure 14. Between-experiment variability in mean urinary urea concentration (mmol/l) from 16 individual experiments measuring faecal N components in cattle fed grass or fodder beet.



Mean total urinary N% followed a similar pattern to urea (Figure 15) reflecting the major contribution of urea to total urinary nitrogen concentration. A diurnal pattern was evident for all feed types when the overall means were examined but within individual experiments the diurnal patterns in grass-fed and fodder-beet fed experiments were inconsistent (Figure 16). In kale-fed cattle, peak N concentrations occurred between 8 and 16 hours after feeding (Figure 16).

There was a higher overall urinary N% in grass- and kale-fed urine compared to fodder beet-fed cattle (Table 10, Figure 15)

Table 10. Mean urinary total N concentration (N%) at four hourly intervals in dairy cattle fed grass, kale or fodder beet.

	Hours after feeding							
Feed type	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**Mean**
Grass†	0.563	0.536	0.464	0.544	0.581	0.580	0.563	0.545
Kale	0.339	0.574	0.622	0.583	0.574	0.424	0.315	0.519
Fodder beet	0.307	0.312	0.348	0.313	0.287	0.294	0.315	0.310

† Data for cattle fed 4pm

Figure 15. Mean urinary N% at four hourly intervals in dairy cattle fed grass, kale or fodder beet.

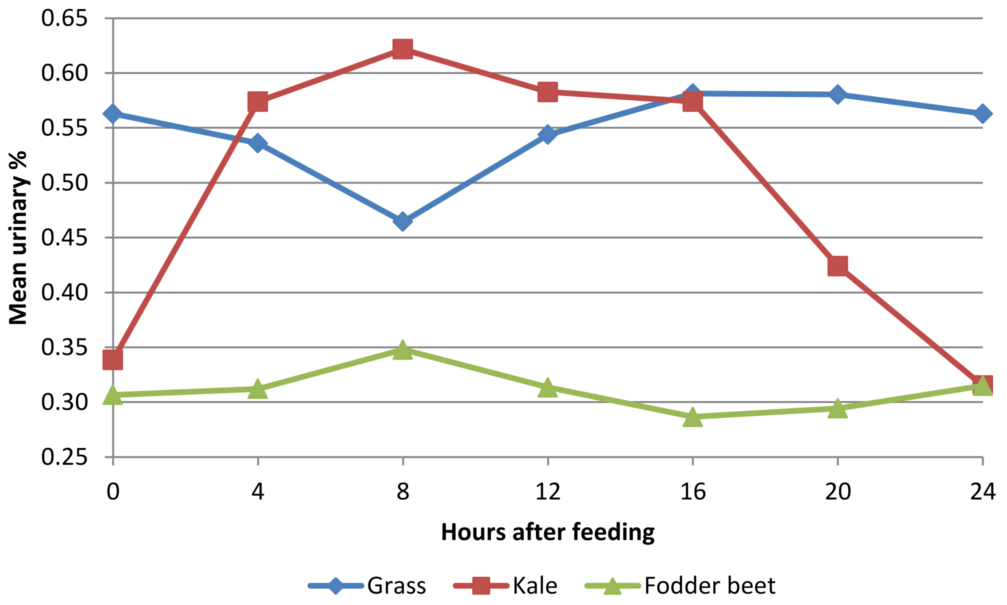
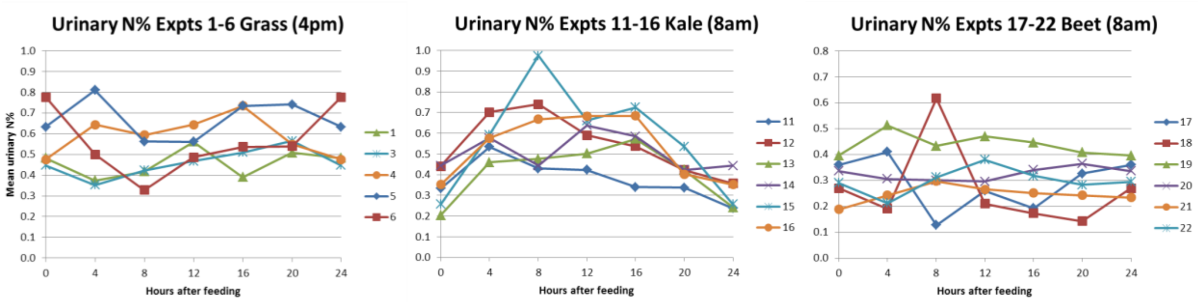


Figure 16. Between-experiment variability in mean urinary N% from 18 individual experiments measuring urine N components in cattle fed grass, kale and fodder beet.



Discussion

This analysis identified diurnal variations in the concentrations of faecal and urinary nitrogen components and these appeared to be associated with time of feeding and the diet offered.

Over a 24 hour period, the concentration of faecal N components varied by about 20% for all feed types (Figures 5, 7 and 9) and concentrations of urinary nitrogen components varied by up to 100% (Figures 11, 13, and 15). These patterns are similar to those reported by other workers. Betteridge et al. (1986) reported a diurnal pattern in frequency of urination, urine volume and urinal N concentration in steers grazing high quality pastures and urinary N concentrations were consistently 2-3 times higher at night than during the day. Similarly, Betteridge et al. (2013), Clark et al. (2010) and Draganova et al. (2010) observed a strong correlation between grazing activity and urination in cattle. Greatest urination frequency and volume occurred during dawn and dusk grazing bouts and following prolonged periods of lying behaviour. Betteridge et al. (2010) observed similar correlations in grazing sheep. Clark et al. (2011) recorded an almost doubling in urine volume and total nitrogen excreted in the hour after feed was offered. In contrast, urinary N concentration peaked 4 hours after feeding, with the delay attributed to ruminal proteolysis and microbial growth in relation to feeding.

The pattern of faecal ammonia and urea concentrations were similar whether fed in the morning (8am) or afternoon (4pm; Figures 1 and 3). Typically, mean faecal NH_3 concentration declined after feeding to reach minimum about 12 hours after feeding (Figure 1). In contrast, faecal urea concentrations increased to maximum between 8 and 12 hours after feeding (Figure 3). This means that in morning-fed animals peak faecal urea concentration would have occurred in late afternoon, whereas in afternoon fed animals it would have occurred during the evening.

However, the diurnal patterns for individual experiments varied widely with some experiments showing a strong diurnal pattern while others had a much flatter diurnal profile. Betteridge et al. (1986) also found wide variation in values between individual steers and days. In the current analysis, experiments with the highest overall nitrogen concentrations generally had the highest peak N concentrations between 8 and 16 hours after feeding while the experiments with low overall nitrogen concentrations had flatter curves with correspondingly lower and earlier peak values (between 4 and 12 hours after feeding). This variability in diurnal patterns and absolute nitrogen concentrations (Figures 2 and 4) made it difficult to define a reliable ‘mean’ curve for this time-of-feeding effect.

There was a clear and consistent difference in faecal and urinary N concentrations between feed types, with grass-fed cattle having the highest N concentration , fodder beet-fed cattle the lowest and kale-fed cattle intermediate (Figures 9 and 15). Furthermore, the between-experiment variability in both diurnal pattern and mean N concentrations was highest in grass-fed cattle, lowest in fodder beet-fed cattle and intermediate in kale-fed cattle.

One potential source of variability between feeds is the crude protein (CP) and water soluble carbohydrate (WSC) content of the feeds (Table 11) which may influence ruminal nitrogen metabolism and, hence, urinary nitrogen excretion. Ryegrass generally has higher CP and low WSC compared fodder beet with low CP and higher WSC concentration. Kale is generally intermediate between these (Table 11). The ratio of CP:WSC in feeds is known to influence ruminal ammonia production and urinary excretion of urea (Miller et al. 2001; Moorby et al. 2006; Cosgrove et al. 2007; Pacheco et al. 2010). For feeds where crude protein intakes are likely to be higher relative to the water soluble carbohydrates (e.g.

ryegrass) there is potential for greater ammonia production in the rumen which is converted to urea and excreted in the urine. Conversely with feeds of low CP:WSC ratio (e.g. fodder beet) rumen-generated ammonia is largely captured by rumen micro-organisms and excretion via urea in urine is reduced. Kale, with moderate CP and WSC falls between these extremes. This pattern is reflected in the magnitude of N concentrations observed in this analysis.

Table 11. Examples of nutritional composition for feeds typical of those in the current studies

Feed	Dry matter %	Crude Protein (%DM)	ME (MJ/kgDM)	Water soluble carbohydrate (% DM)
Ryegrass	15	15-25	11.0	21-28
Kale	15	20	12.5	14-38
Fodder beer	14-20	9-14	12-12.5	20-70

Sources: Easton et al. (2009), Fleming (2003), Ruguho et al. (2010)

In the current analysis, observed differences and variability in diurnal patterns and magnitude of faecal and urinary N concentrations are also likely to reflect differences in feed intake and water intake. Total daily feed intake of grass-fed cattle represented in these experiments was between 16-20 kilograms of dry matter, while for crop-fed cattle it was 8-15 kilograms of dry matter (Gibbs S.J., pers. comm.). The larger water volumes ingested by grass-fed cattle, and the very high rates of intake after the initiation of grazing, resulted in greater urine volumes in that period. Increased water intakes will alter urine and faecal water outflows and reduce concentrations of excreted metabolites. It must be noted that values reported in this analysis are concentrations and, without knowledge of total volumes excreted, we cannot extrapolate these values to total amounts of nitrogen, urea and ammonia excreted.

Conclusions

The current analysis adds to and supports published evidence for diurnal variation in faecal and urinary nitrogen concentration in cattle fed grass, kale and fodder beet. These patterns appear to have been influenced by feeding time. It is likely that the amplitude of diurnal patterns is largely influenced by nitrogen intake thought this cannot be confirmed from the current analysis.

There are clear differences in the concentration of nitrogen being excreted on grass, kale and fodder beet, probably as a result of differences in nutrient composition. The key influence is likely to be through the ratio of crude protein to water soluble carbohydrate as it affects rumen ammonia production and urinary N excretion.

Because of variability between individual and unrelated studies, there is insufficient information to *predict*, with any confidence, diurnal patterns of N excretion based on feeding time or feed composition. Further studies are required to:

1. Characterise diurnal N excretion in grass-fed ruminants in terms of both N concentration and **total N output** to provide more accurate predictive curves based on feed characteristics and intake.
2. Directly compare the effect of morning and afternoon feeding on diurnal patterns of N excretion within controlled experiments.
3. Evaluate diurnal N excretion patterns in set stocked pasture situations which are more typical of commercial sheep and beef operations.

References

Betteridge, K., Andrewes, W.G.K., and Sedcole, J.R. (1986). Intake and excretion of nitrogen, potassium and phosporus by grazing steers. The Journal of Agricultural Science *106*, 393–404.

Betteridge, K., Costall, D., Balladur, S., Upsdell, M., and Umemura, K., (2010). Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture. *Animal Production Science*, 50 (6), 624–629.

Betteridge, K. Costall, DA, Li, FY, Luo, D and Ganesh, S. (2013). Why we need to know what and where cows are urinating – a urine sensor to improve nitrogen models Proceedings of the New Zealand Grasslands Association 75:119-124

Clark, C.E.F., Levy, G., Beukes, P., Romera, A., and Gregorini, P. (2010). Predicting the location of dairy cow urinations. In Proceedings of the European Association of Animal Production Annual Meeting, (Heraklion, Greece), p. 328.

Clark, C., Waghorn, G.C., Gregorini, P., Woodward, S.J.., and Clark, D.A. (2011). Diurnal pattern of urinary and faecal nitrogen excretion by dairy cows fed ryegrass pasture twice daily indoors. Advances in Animal Biosciences *2*, 269.

Cosgrove, G.P., Burke, J.L., Death, A.F., Hickey, M.J., Pacheco, D., and Lane, G.A. (2007). Ryegrasses with increased water soluble carbohydrate: evaluating the potential for grazing dairy cows in New Zealand. In: Proceedings of the New Zealand Grassland Association, p. 179.

Draganova, I., Yule, I., Hedley, M., Betteridge, K., and Stafford, K. (2010). Monitoring Dairy Cow Activity with GPS-Tracking and Supporting Technologies. In 10th International Conference on Precision Agriculture (ICPA), (Denver),

Easton, H.S., Stewart, A.V., Lyons, T.B., Parris, M., and Charrier, S., (2009). Soluble carbohydrate content of ryegrass cultivars. In: Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, 161–166.

Fleming, P. H. (2003). Farm technical manual 2003. Lincoln University. Farm Management Group.

Miller, L.A., Moorby, J.M., Davies, D.R., Humphreys, M.O., Scollan, N.D., MacRae, J.C., and Theodorou, M.K. (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): milk production from late-lactation dairy cows. Grass and Forage Science 56, 383–394.

Moorby, J.M., Evans, R.T., Scollan, N.D., MacRae, J.C., and Theodorou, M.K. (2006). Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.). Evaluation in dairy cows in early lactation. Grass and Forage Science 61, 52–59.

Pacheco, D., Lowe K, MJ, H., JL, B., and GP, C., (2010). Seasonal and dietary effects on the concentration of urinary N from grazing dairy cows. Proceedings of the Australasian

Dairy Science Symposium, Proceedings of the 4th Australasian Dairy Science Symposium, 68–73.

Ruguho, I, Gibbs, S.J., Bryant, R.H., Edwards, G.R., (2010). Intake and feeding behaviour of dairy cows grazing kale and grass at low and high allowances during winter. Proceedings of the Australasian Dairy Science Symposium, Proceedings of the 4th Australasian Dairy Science Symposium, 317–320.