Diurnal urinary excretion of DHP in steers fed *Leucaena leucocephala*

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Introduction

Leucaena (*Leucaena leucocephala*) contains the toxin mimosine which is quickly degraded by rumen microorganisms to isomers of dihydroxypyridine (DHP). DHP is detrimental to animal production, causing reduced thyroid hormones, reduced weight gain, goiter and severe deficiencies in essential minerals (Tsai and Ling 1971; Hammond 1995). There are several methods of testing for exposure to DHP toxicity but the simplest is the colorimetric urine spot test (Graham *et al.* 2013). Several researchers have noted high variability in the excretion of DHP among animals on similar leucaena diets (Dalzell *et al.* 2012; Phaikaew *et al.* 2012) and even in the same animal over sequential samplings (O'Reagain and Shelton 2013). They noted that it was possible to obtain samples with very low DHP in unprotected animals on high leucaena diets, leading to the false conclusion that the animal was successfully degrading DHP in the rumen. This study examined the extent and possible causes of variation of DHP concentration in spot urine samples taken over a 6-week period, including an intensive sampling over a 24 hour period.

Methods

Sixteen mixed breed stall-housed steers (200-300 kg liveweight), previously naïve to *S. jonesii*, were offered diets of 25%, 50% and 100% leucaena/grass at 2.5% body weight for 6 weeks. Leucaena and grass intake was measured daily. On the same day each week a spot urine sample was obtained from all animals between 12pm and 3pm as well as a sub-sample of bulked urine collected over 24-hours. Near the end of the trial period on day 40, one steer from each treatment was placed in a metabolism crate and all urine events sampled over a 24-hour period. The remaining urine was pooled into a bulk collection for each animal. Total DHP (both 3,4-DHP and 2,3-DHP) was determined for all samples by high performance liquid chromatography (Dalzell *et al.* 2012).

Results and Discussion

Weekly spot urine DHP concentrations were significantly correlated to weekly bulk urine DHP concentrations but were a poor predictor of the concentration of the bulk samples (Fig. 1). Weekly spot urine DHP concentrations were highly variable both within and between animals (Fig. 2) with variability greatest in animals fed 100% leucaena in diet.

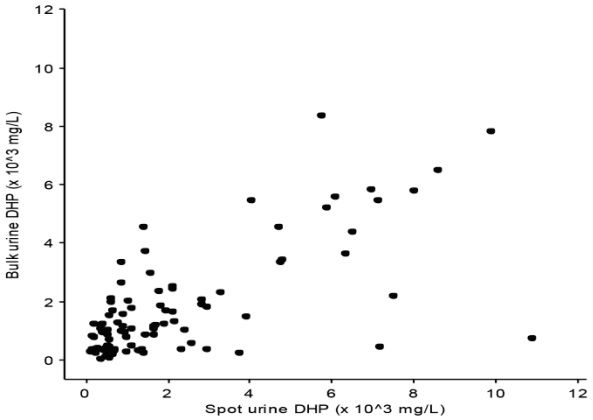

Figure 1. Relationship between spot and bulk urinary DHP concentration

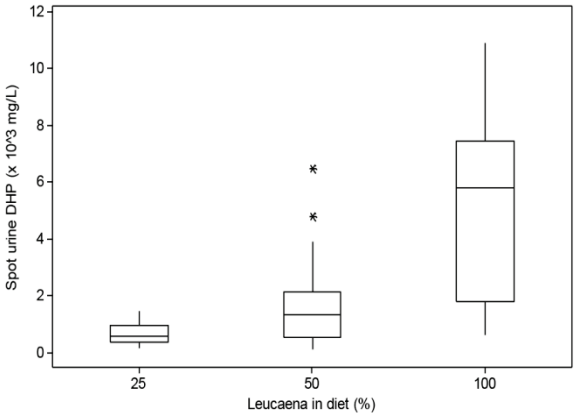

Figure 2. Box and whisker plot for weekly spot urine DHP concentration of 16 steers offered 25, 50 and 100% leucaena over a period of 6 weeks

DHP concentrations in spot urine samples taken on day 40 were also highly variable (Table 1). A t-test indicated that the mean DHP concentration of 22-34 spot urine samples was significantly different to the bulk urine DHP sampled in the same 24 hour period. In addition, bulk and spot urine DHP did not reflect the amount of leucaena eaten by the four animals with animals A and D excreting relatively less DHP then expected compared to animals B and C. This inconsistency was evident even when urine volume of the bulk

Table 1. Summary intake and urinary data collected during 24 hours on day 40.

Animal ID	A	B	C	D
Dietary intake				
Leucaena eaten (kg DM)	1.6	1.7	3	4.5
Leucaena in diet (%)	30	34	48	100
Bulk urine results				
Total urine volume (L)	2.6	3.1	3.1	16.9
DHP concentration (mg/L)	318	1240	2630	234
DHP excreted (mg)	820	3858	8231	3952
Spot urine results				
Number of samples	25	23	22	34
Mean DHP concentration (mg/L)	458 ± 54	853 ± 107	1536 ± 232	701 ± 154
Range (mg/L)	106 - 1180	307 - 1822	470 - 3782	69 - 3466
95% Confidence interval	347 - 569	632 - 1074	1054 - 2018	397 - 1005

sample was taken into account and contrasts earlier findings. However, it is likely rumen bacteria (probably S. jonesii) were actively degrading DHP in animals A and D (J Padmanabha unpublished data). Further analysis of day 40 data (loge transformed) showed that time of day; time since leucaena feeding; time between urination events; and number of urination events were not related to spot urine DHP concentration. Previous work has shown that when cattle are fully protected against DHP toxicity, average herd urinary DHP was < 100 mg/L DHP (Graham *et al.* 2013). Using this threshold, spot urine samples from animals A, B and C would have consistently indicated the animals were unprotected; however, 22% of samples from animal D were <100 mg/L and would have given a false protection status despite a high leucaena intake.

Conclusion

There was significant variation in the DHP concentration of spot urine samples both over the 6 weeks of the trial and within the 24 hours of intensive measurement. The observed variability was most likely due to rumen bacteria actively but differentially degrading DHP in two of the four steers; however other metabolic factors may also have influenced DHP excretion. This variation could result in misleading assessment of the protection status of animals suffering subclinical leucaena toxicity when using the colorimetric test on single samples. This finding supports the recommendation of Graham *et al.* (2013) that ≥ 10 samples from different animals or from repeated sampling of individual animals, is required to accurately assess the toxicity status of cattle herds.

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