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BRIEF COMMUNICATION: The Effect of the Time of Day on Creatinine Excretion from Sheep fed Perennial Ryegrass or Plantain

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Abstract

This study investigated the feeding of plantain (*Plantago lanceolata L.*) or perennial ryegrass (*Lolium perenne L.*) on creatinine excretion from sheep over the day. Eleven castrated Romney-cross male lambs were separated into two groups fed either Plantain (n=five) or Ryegrass (n=six) in an indoor metabolism trial for eight days (three-day adaptation period and five-day collection phase). Urine samples were taken from each sheep at 6am, 9am, 12pm, 3pm and 6pm over 4 days and analysed for concentration of creatinine. Mean creatinine concentration was lower ($p<0.05$) for the plantain group which can largely be attributed to the diuretic effect of plantain. Forage treatment did not influence the quantity of creatinine excreted by sheep per period or per day ($p>0.05$). Time of day and the interaction between forage type and time influenced the concentration and amount of creatinine in urine ($p<0.05$). For both forages, the mean creatinine concentration from the 12pm-3pm period most closely approximated the daily mean creatinine concentration.

Keywords: Creatinine excretion, sheep, diurnal rhythm, plantain, ryegrass

Introduction

Livestock urine can be a major source of nitrate leaching and emissions of N_2O in pasture-based farming systems (Ledgard et al., 2009). Thus, understanding urine Nitrogen (N) excretion patterns is important for research investigating N_2O emissions or nitrate leaching. An animal's daily urinary excretion traits are commonly measured using metabolism crates, this method, however, can be expensive and labour-intensive (Llonch et al., 2016) and does not accommodate grazing experiments.

An alternative method to estimate urine volume and N excretion is the use of creatinine as a urine volume marker in urine spot samples. Previous research has reported creatinine excretion is mostly reliant on liveweight (Cetinkaya et al., 2006; Chizzotti et al., 2008) and largely independent of diet (Dapoza et al., 1999; David et al., 2015; Susmel et al., 1995) which has led to it being identified as a suitable marker in urine. In dairy cattle creatinine excretion has already been used with good accuracy to predict urine volume and other urinary compounds (Tebbe & Weiss, 2018; Valadares et al., 1999), however it has not been widely used for sheep. Maheswaran et al. (2022) found a large variation in urine volume estimated from creatinine concentration in spot samples of urine from sheep and proposed that time of day and forage types used in New Zealand sheep systems may affect creatinine excretion.

The purpose of this study was to investigate whether time of day and/or feeding perennial ryegrass (*Lolium perenne* L.) or plantain (*Plantago lanceolata* L.) influenced traits of creatinine excretion such as creatinine concentration and total excretion over 3-hour sampling intervals during the day.

Materials and methods

Study Design

This study was carried out at Massey University, Palmerston North in New Zealand (Massey University Animal Ethics AEC 23/60). Monocultures of perennial ryegrass (*Lolium perenne* L. cv. Maxsyn) and plantain (*Plantago lanceolata* L. cv. Agri Tonic) were sown in Spring 2023. The study was undertaken during late summer (February) of 2024 using 11 Romney-cross wether lambs (approximately 6 months of age) over a total of eight days. This included a three-day adaptation period in individual pens followed by a five-day collection phase in metabolism crates. All lambs used in this experiment were acclimatised to indoor confinement and handling. On Day one, lambs were balanced for liveweight and allocated to a diet of either perennial ryegrass (n=six) or plantain (n=five). Over the course of the study all lambs were provided water and their allocated forage treatment at 9am and 4pm, *ad libitum*.

Measurements

Lambs were weighed at the beginning and end of the study using a weigh crate. During the collection phase all feed, faeces, water and feed refusals were weighed daily. Urine was weighed at 6am, 9am, 12pm, 3pm and 6pm and two subsamples were collected at each time (one non-acidified and one acidified with 98% sulphuric acid). Samples were also taken of the feed (1 sample per forage per day), feed refusals (pooled for each forage over whole experiment) and faeces (pooled by animal ID). All the samples were immediately frozen before being freeze-dried and ground and sent to the Massey University Nutrition lab for chemical analysis.

Chemical analysis

The acidified urine samples were analysed for N content using the Dumas method (AOAC 968.06) and the non-acidified samples were analysed for creatinine using colourimetric methods with Picric acid.

Statistical Analysis

The quantity of creatinine excreted from each sheep for the different times was determined by multiplying the concentration of creatinine in the urine samples with the corresponding urine volume for that time and animal. The concentration (µmol/L), and quantity of creatinine excreted in each sample (µmol) was analysed using a repeated measure analysis model on SAS 9.4 version using the proc GLM function, with day as the repeated value. The amount of creatinine excreted over the 6pm-6am interval (12 hours) was divided by 4 for comparison to the other 3-hour intervals. In the models used forage treatment and time of day were fixed effects, sheep and day were random effects.

Results and discussion

Dry matter intake was similar ($p>0.05$) for the sheep fed plantain and the sheep fed ryegrass with means of 1.4 kg and 1.26 kg er day respectively.

Creatinine Concentration

The concentration of creatinine in urine was affected by treatment ($p<0.05$) and sampling time ($p<0.01$), though the interaction between treatment and time was not significant ($p>0.05$; Figure 1). For the plantain group, creatinine concentration was greater ($p<0.05$) for the 6am-9am period while all other periods were not different ($p>0.05$) to each other. For the ryegrass group, creatinine concentration was lower at 3pm-6pm and greatest at 9am-12pm ($p<0.05$). The mean daily creatinine concentration for plantain and ryegrass groups was 1168± 407 and 2923± 371 µmol/L respectively. Meanwhile the mean daily urine volume for both groups was 3.81±0.22L for plantain and 1.85±0.20L for ryegrass. For both groups urine volume excreted per 3 hours was highest for the 3pm-6pm time (Figure 1).

Creatinine Excretion

The amount of creatinine excreted in urine was not affected by forage type ($p>0.05$), though both the time of day and interaction between treatment and sampling interval were significant ($p<0.01$). On average, sheep in the plantain and ryegrass groups excreted 0.343 ± 0.013 and 0.309 ± 0.012 mmol/kgLW$^{0.75}$ per day respectively, these means were not statistically different ($p>0.05$).

Table 1. Least Square means of creatinine excreted (µmol) over each 3-hour period and the overall 3-hour mean by treatment.

	Plantain		Ryegrass			
Time	**lsmeans**	**SE**	**lsmeans**	**SE**	**P-values**	
6pm-6am*	611^{ab}	35.5	491^{a}	32.3	Treatment	0.4016
6am-9am	584^{a}	34.5	682^{c}	32.3	ID(Treatment)	<0.0001
9am-12pm	549^{a}	35.5	526^{a}	31.5	Treatment*Time	0.0044
12pm-3pm	694^{b}	34.5	558^{ab}	31.5	Time	0.0004
3pm-6pm	692^{b}	34.5	633^{bc}	31.5	Day*Time	<0.0001
Mean per 3 hrs	599	46.5	551	45.4	Treatment	0.4803

*The amount of creatinine excreted was divided by 4 to account for longer interval length.

^{a,b,c} Superscripts denote significant ($p<0.05$) differences between values within columns using LSD test.

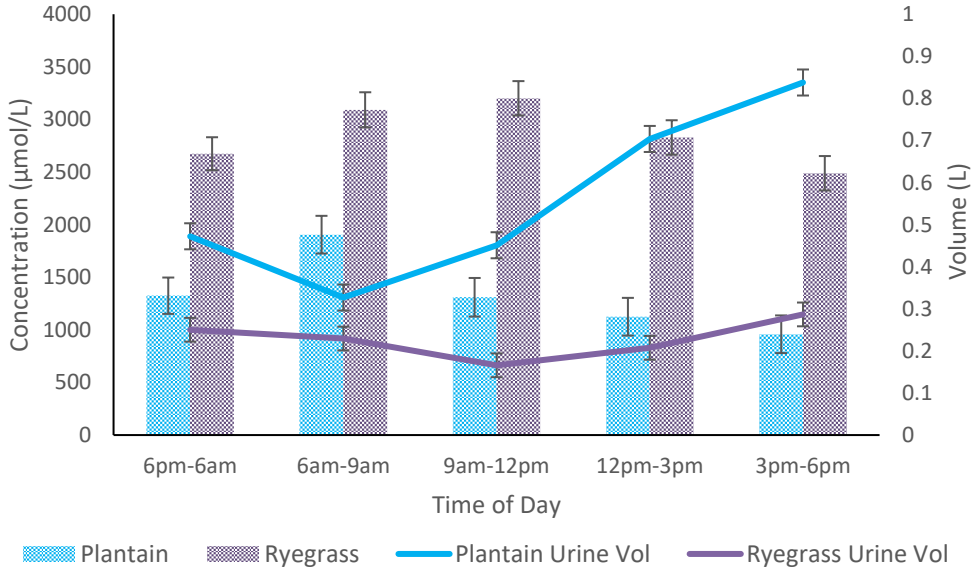

Figure 1. Mean concentration of creatinine (µmol/L; bars) and urine volume (L; lines) by forage treatment for each measured period. Error bars represent the standard error between sheep and days. (Urine volume for the 6pm-6am period has been divided by 4 for comparison purposes).

In the current study, feeding plantain did not significantly affect the amount of creatinine excreted in sheep urine in each period ($p>0.05$, Table 1) but it did affect creatinine concentration. Plantain feeding increased the mean volume of urine, exhibiting a diuretic effect. In previous studies with sheep, feeding plantain has resulted in a wide range of increases in urine volume when compared to perennial ryegrass from 0.5-1.9L/d without significant effect on total excretion of osmols (McGusty, 2017; O'Connell et al., 2016). In our study lambs fed plantain produced 1.9L/d additional daily urine aligning with the 1.9L/d difference observed in the first trial by McGusty (2017) and much greater than the mean difference of 0.5L/d from days 2-6 of the trial by O'Connell et al. (2016). Both of the previous studies tried to balance water intake between the groups of sheep but only O'Connell et al. (2016) successfully balanced water intake which may contribute to why there was a much smaller difference in urine volume. In the present study, lambs offered the plantain had a ~1.6L/d greater total water intake (from forage and water; data not shown) than ryegrass lambs.

The time of day influenced both creatinine concentration and excretion from sheep fed either plantain or perennial ryegrass. The greatest mean creatinine concentration was observed during the 6am-9am interval for the plantain group and 9am-12pm for the ryegrass group (Figure 1). This is in disagreement with the findings of Dos Santos et al. (2018) who found creatinine concentration in the urine of sheep fed 50:50 ratio of sorghum silage and concentrate, was higher in nocturnal periods. Whilst for both groups the lowest mean creatinine concentration was at the 3pm-6pm interval (Figure 1), which agrees with Cosgrove et al. (2017) as dairy cattle fed ryegrass also had lower creatinine concentrations in the late afternoon. Notably, the greatest creatinine concentrations corresponded with the lowest urine volumes for each forage and vice versa suggesting the variation in creatinine concentration may be heavily dependent on changes in urine volume.

The mean quantity of creatinine excreted per 3 hours by the plantain sheep was 599 µmol, this value was most closely approximated by the 6pm-6am mean of 611 µmol creatinine. For the ryegrass group the daily mean amount of creatinine excreted per 3 hours was 551 µmol, most closely approximating the 12pm-3pm mean of 558 µmol though this mean was only significantly ($p<0.05$) different to the 6am-9am period. Similarly, in a previous study using sheep fed sorghum silage and concentrate and taking measurements every 4 hour intervals, the overall mean amount of creatinine excreted per 4 hours was closest to the mean amounts excreted during the 4am-8am and 12pm-4pm intervals (Dos Santos et al., 2018).

When it comes to creatinine concentration, it appears that for both groups the concentration of creatinine in urine from the 12pm-3pm period most closely approximates the daily mean (Figure 1). So, it may be more accurate to collect urine spot samples in this period when aiming to estimate urine volume using predictive equations based on creatinine concentration. Though the effect of forage type and time of day should be further investigated, with focus placed on the timing of feeding and the relationship between urine output rate and creatinine concentration.

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