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**The relationship between plasma and saliva urea  
nitrogen in grazing red deer calves**

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A dissertation  
submitted in partial fulfilment  
of the requirements for the Degree of  
Bachelor of Agricultural Science with Honours

at  
Lincoln University  
by  
Ella Wilson

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Lincoln University

2022

Abstract of a dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science with Honours.

The relationship between plasma and saliva urea  
nitrogen in grazing red deer calves

by

Ella Wilson

I evaluated the relationship between saliva urea N (SUN) and plasma urea N (PUN) in red deer calves ( $n = 23$ ) over five months, with the objective of creating an easy tool to quickly evaluate circulating urea nitrogen concentrations. The experimental design was a 2x2 factorial arrangement different seasonality phenotype (High and Low) and two different forage based diets, a medium-quality diverse treatment (Diverse) or a low-quality perennial ryegrass-white clover treatment (PRG) offered *ad libitum*. Blood and saliva samples for determination of PUN and SUN were evaluated monthly at civil dawn between April and September of 2022. A moderately strong relationship between PUN and SUN was identified by simple linear regression ( $R^2 = 0.65$ ,  $P < 0.001$ ). The interaction between diet and date of sampling had a significant effect on the relationship between SUN and PUN ( $P < 0.001$ ). This interaction is likely explained by the seasonal variation of crude protein (CP) content of the diet. The use of a mixed model procedure improved the model fit increasing the adjust  $R^2$  by 0.12 and reducing the root mean square error by 0.341. There was no effect of seasonality on prediction estimates of PUN from SUN although a tendency for sex by seasonality interaction was observed ( $P = 0.09$ ). Therefore, evaluation of the diet and date of sampling can be used to improve reliability of the prediction estimates of PUN from SUN in red deer calves. Further investigation into the effects of significant factors on this relationship is required to improve the reliability of the model, before SUN can be used to predict PUN.

**Keywords:** Saliva urea nitrogen, plasma urea nitrogen, deer, seasonality, date, diet, gender

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# Chapter 1

## Introduction

The New Zealand agricultural industry faces surmounting pressure to reduce N pollution into water ways (Duncan, 2014; Ministry for the Environment, 2021; Scarsbrook, 2015). Nitrogen emissions from ammonia ( $\text{NH}_3$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) account for around 12% of New Zealand's total greenhouse gas emissions, and 21% of total greenhouse gas emissions from agriculture (as of 2018) (de Klein, 2020; van der Weerden, 2017; Wecking, 2020). While the N emissions are a reasonably small component of New Zealand's total greenhouse gas emissions, they are important as  $\text{N}_2\text{O}$  is approximately 300 times more effective at trapping heat in the atmosphere compared with carbon dioxide ( $\text{CO}_2$ ). Nitrous oxide also has a greater longevity, remaining in the atmosphere for  $\sim 150$  years (Wypych, 2017) compared with methane ( $\text{CH}_4$ ), which remains in the atmosphere for  $\sim 12$ -15 years (Doble, 2007), and  $\text{CO}_2$ , which can be present in the atmosphere for  $\sim 5$ -200 years (Inman, 2008). Therefore, reduction of  $\text{N}_2\text{O}$  emissions are needed to help reduce NZ agricultural greenhouse gas footprint.

In the New Zealand agricultural context, the main sources of  $\text{N}_2\text{O}$  emission are urinary and faecal N excreted by grazing ruminants, as well as synthetic N fertiliser such as urea (de Klein, 2020; van der Weerden, 2017; Wecking, 2020), which is commonly and excessively used as pasture fertilizer. The latter being closely related to the superlative inefficiency of N utilization by grazing ruminants in these temperate systems, which rarely goes below 70% (Castillo et al., 2001). This means that at least 70% of the N ingested is not utilized to support animal production (e.g., milk, live weight gain), and is excreted, mainly (over 60%) as urinary nitrogen (UN) (Kebreab et al., 2001; Gregorini et al., 2010a). It has been reported that in pasture-based production systems, approximately 82% of UN is discharged onto pastures (Oudshoorn et al., 2008; Clark et al., 2010). Urinary patches are highly concentrated, containing between 700 and 1200kg N/ha (Moir, 2016). Urinary N urea rapidly breaks down into  $\text{NH}_3$ ,  $\text{NO}_3^-$  (nitrate), and  $\text{N}_2\text{O}$ . These highly concentrated pollutants have a significant leaching risk, particularly during the winter period when soils are saturated and plant uptake of N is limited due to low plant growth rates (Chapman, 2020; Moir, 2016). From this UN around 20–30% is leached and 2% transformed to  $\text{N}_2\text{O}$  (IPCC, 2006).

Excessive N in waterways can result in eutrophication, which leads to losses of ecosystem services and biodiversity. Eutrophication promotes algal blooms, which blocks light from penetrating the water and therefore reduces the rate of photosynthesis of aquatic plants having run on effects on oxygen production. This can result in a hypoxic zone resulting in the death of aquatic creatures in the area

and a subsequent loss of ecosystem services. Such a negative impact could impair New Zealand's ability to fulfil its responsibilities under global convention such as the Kyoto Protocol and the Ramsar convention (Schallenberg et al., 2013). There is also a possible association of increased risk of colorectal cancer from high levels of NO<sub>3</sub> in drinking water (Schullehner et al., 2018). These externalities impair the ability of temperate pastoral livestock production systems to meet the United Nations sustainable development goals 6 (Water quality), 9 (Industry innovation), and 12 (Responsible production). These alarming values confirm the need to explore strategies to estimate and reduce the amount of N flowing through grazing ruminants and respond to the political and public pressures on pastoral livestock production systems.

Since its beginnings in the 1970's, deer farming in New Zealand has had a lower environmental impact compared to the dairy and sheep/beef industries. However, and due to the linear relationship between herbage and N intake with N excretion, as the industry rapidly expands (number of animals) and productive systems intensify under greater market demand or displacement by the dairy industry, significant consideration needs to be given to the health and sustainability of the environment (Dinnes, 2002; de Klein, 2003). Overseas buyers, especially those in Europe, are increasingly concerned with both animal welfare and negative environmental impacts of intensive pastoral livestock production systems (Hyland, 2022). Therefore, considering these factors is not only important for sustainability, but also long-term profitability of the New Zealand deer farming industry.

Despite the obvious concerns, the deer industry's N pollution contribution is not entirely clear due to the limited data available. Such a limitation relates to the nature of the deer production systems and the very nature of the animal in question. Deer, as not yet domesticated animals, are not easily accessible and can be difficult to handle to collect urine samples from, as compared with cattle. (Higgins, 2016; Orsonneau, 1992). In comparison, the dairy industry has a clear understanding on both the overall N leaching potential of the of each farm system (Powell, 2015), as well as differences in the N leaching potential of individual animals (Spek, 2013). Again, this understanding is due to the relative ease of urine sampling in dairy cattle. Therefore, the need to easily sample deer to estimate UN becomes imperative to evaluate current and future mitigation practices in term of N pollution of deer production systems in New Zealand.

The collection of urine or milk urea N for estimations of UN excretions is not an easy procedure in all ruminant species and alternative bodily fluids such as blood and saliva can be considered for species such as sheep, deer, goats or male cattle. In domestic or farmed red deer (*Cervus elaphus*), venipuncture is used to measure blood metabolites, such as plasma urea N (PUN), but this practice requires several skilled handlers and can be dangerous and sometimes stressful when blood samples

are collected from larger stags, spikers, or hinds (Carragher, 1997). Urea is known to equilibrate across fluids, enabling relationships between UN urea and other fluids such as milk urea N, saliva urea N, or blood urea N to be established (Marshall, 2020). PUN concentrations has been shown provide an accurate indication of UN urea (Bartle, 1986; Spek, 2013). After urea enters the body, it is synthesised before it rapidly equilibrates throughout bodily fluids (Dall Orsoletta, 2020) due to osmotic equilibrium (Bhave, 2011). The relationship between UN urea and other bodily fluids is typically linear and largely reflects the protein content of the diet (Dall Orsoletta, 2020). Moreover, urea N equilibrium between blood plasma and saliva is used in human medicine as a non-invasive test for nephropathic patients, as well as patients with chronic kidney injury, renal failure and as a test for kidney disease in children (Bilancio, 2018; Kovalčíková, 2018; Lasisi, 2016; Raimann, 2016; Renda, 2017; Shannon, 1977). Although this relationship seems straightforward, it has not been validated in cervids (Bilancio, 2018; Kovalčíková, 2018). Consequently, understanding, testing and validating this link allows for a non-invasive estimation of UN urea concentration that can be used for an easier and more rapid test of large numbers of animals (Bilancio, 2018; Kovalčíková, 2018; Marshall, 2020) and predictions of UN urea excretion (Nousiainen, 2004). The latter, with a potential use for selection and breeding of animals with potential low UN excretion reported for dairy cows by (Spek, 2013; Beatson et al., 2019; Marshall et al, 2020).

Based on the above-mentioned evidence, I hypothesise that saliva urea N (SUN) and plasma urea N (PUN) of red deer calves are as in other animals, linearly related. Therefore, and with the objective of taking the first step to create an easy tool to quickly evaluate large number of deer for UN concentration estimates, the main objective of my research is to evaluate the relationship between SUN and PUN in red deer calves. Secondary, I aim to test this relationship in calves selected for different seasonality phenotype in autumn and winter offered two different forage based diets of contrasting diversity and nutritive value. Using differing conditions will allow for a better analysis of the true relationship between PUN and SUN. By testing and accepting this study's hypothesis, SUN could present a more practical, animal friendly and inexpensive tool to evaluate the interactions between forage, N metabolism, and animal phenotype in growing red deer.

## Chapter 2

### Materials and Methods

#### 2.1 Experimental Site

The experiment was conducted at Lincoln University Research Farm, Deer Unit (43°38'57''S, 172°27'01''E), between April and September 2022. Methods described in this dissertation were approved by the Lincoln University Animal Ethics Committee prior to the initiation of the experiment (AEC 2022\_2022-07).

#### 2.2 Animals and Treatments

Twenty three weaned red deer calves of mixed gender were grouped into a 2x2 factorial arrangement of amplitude of the annual cycle of growth during the first year of life (seasonality) and diet, based on nutritional treatment level. The phenotype for amplitude of the annual cycle of growth was determined using a tissue sample. Animals were paired and blocked into two groups (n = 11, n = 12) based on their seasonality, liveweight, and gender. Each group was randomly assigned a dietary treatment either a medium-quality diverse treatment (diverse) or a low-quality ryegrass-white clover treatment (PRG x WC). During winter months when pasture growth was dormant, both pasture type was supplemented with baleage corresponding to the diet, the diverse diet received a lucerne (*Medicago sativa*) baleage and the PRG x WC diet received perennial ryegrass-based baleage. The experimental unit in this trial is the animal at each point in time because the use of blocking or grouping is not practical given the area available. Animal welfare risks and undue stress of break feeding deer in small pens and small areas also reduces the practicality of blocking/grouping.

#### 2.3 Herbage and Feed Allocation

Deer calves in the PRG treatment rotationally grazed a sward of perennial ryegrass (*Lolium perenne* L.), italian ryegrass (*Lolium multiflorum*), and white clover (*Trifolium repens*), whereas the their counterpart in the diverse treatment grazed a diverse sward, that included perennial ryegrass (*Lolium perenne* L.), italian ryegrass (*Lolium multiflorum*), tall fescue (*Festuca arundinacea*), meadow fescue (*Festuca pratensis*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), lucerne (*Medicago sativa*), chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*) and radish (*Raphanus sativus*). The pastures were established in the Spring of 2021 across three adjacent paddocks each 1.4 ha in size. The PRG pasture comprised one third of each paddock and the diverse pastures comprised the remaining two thirds of each paddock. The placement of ryegrass and diverse areas alternated across each paddock to account for variation of soil types and fertility and prevent

paddock variation affecting plant performance. These pastures were originally established for a previous experiment in which there were two forms of a diverse dietary treatment and this additional area was utilised in the current treatment to account for the cool season dormancy of the diverse plant species previously specified.

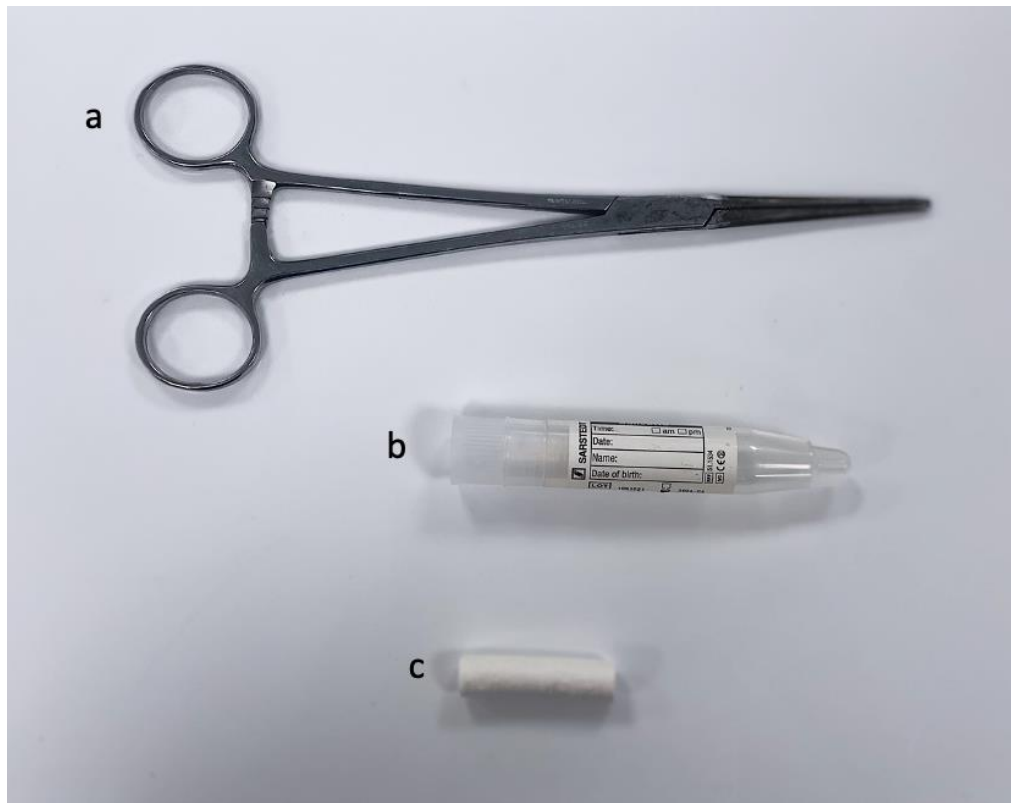
Each third of the paddock (~0.5 ha) was allocated either fortnightly or monthly behind a temporary electric break fence consisting of five reels mounted to a steel real stand to maximise break size and prevent any undue stress associated with using temporary fencing equipment. The animals were also supplemented with the previously described baleage *ad libitum* during periods of pasture deficit, from July until September. The animals grazed the paddocks (n = ~11 per treatment) from April until mid-September, when the final data measurements were collected. Following weaning and blocking, calves were weighed, saliva and blood sampled at dawn (civil twilight) for baseline levels of plasma PUN and SUN,. They were then placed on their dietary treatment and given 30 days for dietary acclimatisation. Herbage mass of pastures were evaluated by cutting all plant material within a 0.2 m<sup>2</sup> quadrat to ground level and drying in a 60 °C oven until achieving a constant weight. Within each treatment, 10 quadrats were collected both pre and post grazing to estimate pasture disappearance of each group.

Plant chemical composition and botanical components were evaluated by collecting ~ 10-20 random hand-grab samples of herbage cut to grazing height (~ 3 cm), pre-grazing. Baleage was also evaluated for collecting random hand-grab samples of each bale as it was allocated to each dietary treatment. Pasture and baleage hand-grab samples were mixed and sub-sampled for dry matter (DM%), botanical and chemical composition. Pasture and baleage hand-grab samples were bulked and separated into three sections to determine DM% (oven-dried at 60 °C until achieving a constant weight), chemical composition and botanical composition. Botanical components were sorted and oven-dried to calculate relative abundance in the sward. The third sample was frozen and stored until freeze-dried, ground through a 1 mm centrifugal mill (ZM200 Retsch GmbH; Haan, Germany) and analysed for chemical components (acid detergent fiber: ADF, neutral detergent fiber: NDF, organic matter: OM crude protein: CP and water soluble carbohydrates: WSC) using near infrared spectrophotometry (NIRS. Model: FOSS NIRS Systems 5000, Maryland, USA). Calibration equations for predicting WSC, CP, ADF, NDF and OM of pasture samples were developed previously. The R<sup>2</sup> values for CP, OM, WSC, NDF and ADF of were all above 0.9 and all samples were within the calibration range.

## 2.4 Animal Management, Sampling and Measurements

Liveweight was continuously measured each fortnight (results will be reported in a separate dissertation) while saliva and plasma measurements were collected from each animal every four weeks throughout the experimental period. Animal measurements were undertaken at civil twilight before they had risen for their dawn grazing bout to reduce the variation of ruminal fill on liveweight – and reduce the need for fasting the animals prior to weighing. Blood samples were used for a further experiment evaluating plasma melatonin and serotonin levels in response to the two seasonally divergent lineages which are responsive to daylight. Calves were mustered from their paddocks and held in two groups based on their diet groups undisturbed in dark-room pens for ~30 minutes prior to sampling. The diverse diet group were sampled first, to minimise variation, in groups of 3-4. They were then held in the yards while the PRG animals were sampled, using the same method. Following sampling, animals were returned to their next forage allocation. Liveweight gain was measured fortnightly, while blood samples (plasma urea N) and saliva samples (saliva urea N) were evaluated monthly between April and September of 2022, the first measurements in April were excluded from these analyses all allow a 30-day dietary acclimatisation period.

Saliva sampling was conducted using salivette tubes (SARSTEDT, Germany). Briefly, the 38 × 10 mm cotton dental swab was taken from the salivette tube and held in the saliva pocket in the side of the mouth of the deer using a long pair of clamping forceps, until the swab was saturated. Equipment used for saliva sampling is shown in Figure 1. After saliva collection, the swab was placed back into the holding container and placed on ice until centrifuged at 4000 g for 15 minutes at 4° C. Blood samples were collected using 20 gauge needles into 10 mL Li heparin-coated vacuette via the jugular vein and immediately stored on ice. Following sample collection, blood and saliva were transferred to the laboratory where blood samples were centrifuged at 3000 g for 15 minutes at 4° C and the resulting plasma was collected into 2 mL Eppendorf tubes and stored at -20° C until analysed. Both plasma and saliva urea nitrogen were evaluated by Randox RX Daytona analyses (Randox Laboratories Ltd, Crumlin, UK).



**Figure 1.** Equipment used during saliva sampling, (a) forceps, (b) salivette tubes and (c) the cotton dental swab

## 2.5 Statistical Analysis

Measurement variables, PUN and SUN, were analysed in two ways. First, samples were grouped into one large dataset, independent of sampling time, dietary treatment and animal phenotype, and a linear regression analysis with a 95% confidence interval was used to evaluate the relationship between PUN and SUN. Secondly, PUN and SUN were analysed using a Generalised linear mixed effects model with diet, phenotype, gender and date of sampling as fixed effects and the individual animal as the random effect, using the “lme” function of the lme4 package (Bates, 2015) of R (r Core Team, 2018, v. 3.4.4.). Interactions between gender, date of sampling and diet were evaluated and included in the final model if significant ( $P < 0.05$ ) and ANOVA of the model was determined using the “car” package. Least square means were generated using the “emmeans” package (Lenth, R.V. Using Lsmeans. CRAN. 2018. (Bates, 2015) of R (r Core Team, 2018, v. 3.4.4.). Upon significance of the ANOVA, means were separated using pairwise contrasts. Differences were declared significant if  $p < 0.05$  and tendencies were  $0.05 < P < 0.1$ .

## Chapter 3

### Results

#### 3.1 Diet Composition and Nutritional Value

Botanical composition of herbage offered at each dietary treatment is presented in Table 1.

**Table 1.** Botanical composition of herbage of each dietary treatment (Diverse and PRG) offered red deer calves of different phenotype for growth pattern.

Treatment	Grasses	Legumes	Forbs	Brassicas	Weeds	Dead Material
<b>Diverse</b>	25.9%	20.7%	8.2%	13.4%	2.3%	29.5%
<b>PRG</b>	24.3%	22.1%	0.0%	0.0%	17.8%	35.7%

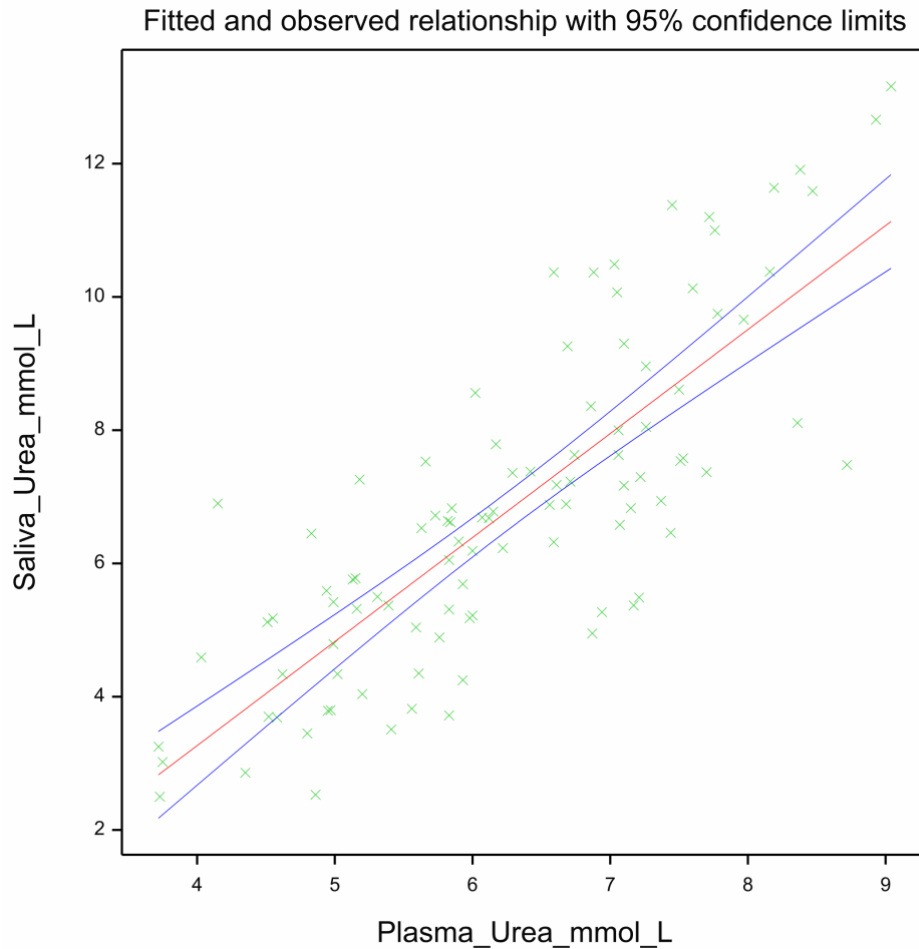
Dry matter content of herbage and baleage in PRG was 34% greater than diverse equivalents. The Diverse herbage had a greater feeding value than PRG, with a 6% lower content of weeds and 16% lower content of dead material. The Diverse pasture had a 17% greater content of crude protein and 31% greater WSC than PRG, with the greatest magnitude of difference between July and September. The herbage in the Diverse pasture also had 4.5% greater metabolisable energy content (MJ ME/kg DM), as compared to herbage offered to the calves in PRG treatment. Digestibility was similar between treatments. The digestibility of the diverse baleage was 7% lower than the PRG one. The digestibility of the Diverse herbage was 6% higher than the PRG herbage. Protein content of diverse baleage was 33% greater than PRG, while the Diverse NDF content was 13% lower than PRG.

**Table 2. Chemical composition and nutritive value of the herbage and baleage offered to growing red deer calves selected for different phenotypes (divergent amplitude seasonal growth) under two nutritional levels treatments, either 'Diverse' or 'PRG', respectively given by either a low-quality ryegrass-white clover swards and baleage or a medium quality diverse sward and lucerne baleage.**

Variable	May – June				June – July								July – August								August – September				
	Diverse	SE	PRG	SE	Diverse	SE	Diverse Baleage	SE	PRG	SE	PRG Baleage	SE	Diverse	SE	Diverse Baleage	SE	PRG	SE	PRG Baleage	SE	Diverse Baleage	SE	PRG Baleage	SE	
<b>Nutritive Value</b>																									
<b>Protein g/kg DM</b>	13.41	0.30	14.06	0.54	16.26	0.25	15.28	1.37	13.50	<0.01	8.49	0.82	21.18	0.03	15.69		22.13	0.50	15.21	0.61	16.62	0.37	8.39	0.41	
<b>NDF g/kg DM</b>	44.29	1.46	55.97	1.01	41.55	0.74	56.62	2.81	55.99	0.67	63.57	3.76	41.98	0.14	53.41		36.59	2.42	49.96	1.52	47.63	1.80	68.45	0.03	
<b>ADF g/kg DM</b>	29.53	0.66	33.23	0.69	27.32	0.34	44.16	1.94	31.25	0.36	35.89	2.19	22.58	0.01	42.09		22.32	1.60	31.70	1.16	33.40	3.84	40.33	0.45	
<b>Digestibility (DOMD) g/kg DM</b>	60.80	0.48	56.08	0.83	65.23	0.53	45.52	3.05	61.30	0.24	57.74	4.33	71.96	0.77	56.82		69.54	0.54	66.41	1.42	61.16	6.51	51.14	1.26	
<b>MJ ME/kg DM</b>	9.63	0.12	9.17	0.09	10.12	0.02			9.71	0.01	66.41		11.02	0.02			10.79	0.22							
<b>OM g/kg DM</b>	93.04	0.56	93.46	0.18	91.67	0.13	92.41	0.02	93.01	0.18	94.42	0.07	90.69	0.06	91.83		90.92	0.53	90.46	0.39	90.73	0.44	93.59	0.35	
<b>WSC g/kg DM</b>	14.46	0.49	8.83	0.25	16.16	0.06			12.87	0.11			21.63				14.43								
<b>DM g/kg DM</b>	25.00	6.79	29.10	8.03	24.30	7.13	29.20	0.44	40	6.65	66.40	0.71	20.30	68.31	41.40	0.03	17.80	3.55	56.90	0.44	47.80	1.04	65.9	0.37	
<b>Average DM Available per Animal per Day</b>	19.97		21.68		12.13		<i>ad libitum</i>		12.52		<i>ad libitum</i>		20.06		<i>ad libitum</i>		4.48		<i>ad libitum</i>		<i>ad libitum</i>		<i>ad libitum</i>		
<b>Average % DM Consumed</b>	43%		45%		53%				15%				73%												

### 3.2 Relationship Between Plasma Urea N and Saliva Urea N

The mean of PUN and SUN were similar, 6.26 and 6.79 mm/L, respectively, showing a linear relationship ( $SUN = -2.978 + PUN \times 1.561$ ; Figure 2.), with a Pearson correlation coefficient of 0.8 and an  $R^2$  of 0.65 ( $P < 0.001$ ). The linear relationship between PUN and SUN is presented in Figure 2. Evaluation of the relationship between PUN and SUN, using the mixed model described previously, improved model fit and increased the adjusted  $R^2$  from 0.65 to 0.77 and reduced the root mean squared error from 0.734 to 0.393.



**Figure 2.** Relationship between SUN and PUN for growing red deer calves selected for different phenotypes (divergent amplitude seasonal growth) under two nutritional levels treatments, either 'Diverse' or 'PRG', respectively given by either a low-quality ryegrass-white clover swards and ryegrass based baleage or a medium quality diverse sward and lucerne baleage.

The mean difference between SUN and PUN is presented in Table 3

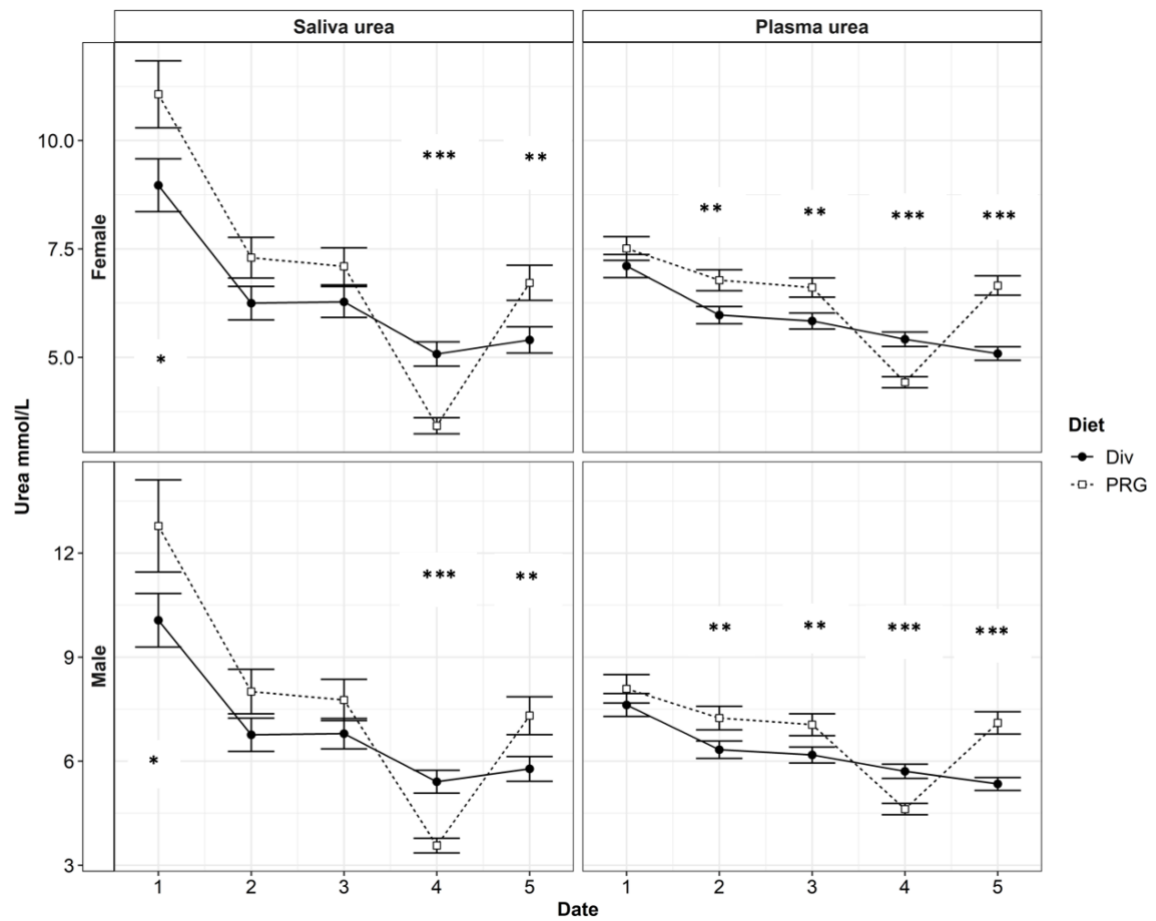
**Table 3.** Mean differences ( $\pm$  standard error) between saliva urea nitrogen (SUN) and plasma urea nitrogen (PUN) of high or low seasonality red weaner deer fed either a perennial ryegrass-based diet (PRG) or diverse pasture species diet (Diverse). Significance of interaction terms between diet and month (D x M) and gender and seasonality (G x S) are also presented.

	<i>P-value*</i>							
	PRG	Diverse	Diet	Month	Gender	Seasonality	D x M	G x S
<b>SUN</b>	6.42 $\pm$	6.39 $\pm$	0.02	***	0.23	0.75	***	0.09
<b>(mmol/L)</b>	0.231	0.187						
<b>PUN</b>	6.37 $\pm$	5.97 $\pm$	**	***	0.21	**	***	0.09
<b>(mmol/L)</b>	0.155	0.114						

\*  $P < 0.01 = **$ ;  $P < 0.001 = ***$ .

The Diverse diet reduced PUN compared with that of the PRG treatment, while dietary means of SUN were similar between diets. A significant interaction between Diet and Month was detected for both PUN and SUN ( $P < 0.001$ ; Table 3). In the month of August the PRG diet reduced both PUN and SUN compared with the Diverse treatment. In September, both PUN and SUN increased in the PRG diet and were greater than that observed from calves given the Diverse diet. The SE for SUN was twice the standard error of PUN (Table 3) as depicted in Figure 3, which presents the greater variability of SUN values compared to PUN. The effect of gender or seasonality (calf phenotype) on either PUN or SUN was not significant ( $P > 0.05$ , Table 3), but a tendency for a gender  $\times$  seasonality interaction was identified ( $P = 0.09$ ).

High seasonality hinds had lower PUN (6.29 and 5.64  $\pm$  0.221 mmol/L, spikers and hinds respectively) and SUN (7.0 and 6.0  $\pm$  0.353 mmol/L, spikers and hinds respectively) concentration compared with the High seasonality spikers. While PUN (6.42 and 6.39  $\pm$  0.171) and SUN (6.35 and 6.35  $\pm$  0.234) of the low seasonality hinds and spikers were similar ( $P > 0.1$ ). Month and Diet had a significant influence on the relationship between SUN and PUN. Including the interaction effects of Month and Diet can improve the  $R^2$  to  $\sim 0.75$  ( $P < 0.001$ ). Urea N levels in all categories of the low-quality ryegrass-white clover treatment were significantly lower in August (Month 4). The PUN levels of deer on the Diverse diet did not decline significantly in August. For all treatments, there was an overall decrease in urea N levels throughout the trial, with the lowest urea N levels in August and September (month 4 and month 5). The N content of SUN and PUN over time as grouped by gender is presented in Figure 3.



**Figure 3.** The nitrogen content of plasma urea nitrogen (PUN) and saliva urea nitrogen (SUN) in red weaner deer each month between April and September. Date refers to each monthly sampling between April and September (labelled 1:5, respectively). Significance of Diet (either a diverse pasture: Div, or a ryegrass-based diet: PRG) is indicated by  $P < 0.05$ : \*,  $P < 0.01$ : \*\*,  $P < 0.001$ : \*\*\*.

## Chapter 4

### Discussion

#### 4.1.1 The relationship between saliva urea N and plasma urea N

The objective of my research was to evaluate the relationship between SUN and PUN in red deer fawns of high and low seasonality growth pattern phenotype fed either a moderate quality, diverse sward or a low-quality ryegrass-white clover sward. The results of this study support my hypothesis, as they indicate that there was an acceptable positive linear relationship between SUN and PUN ( $P < 0.001$ ,  $R^2 = 0.65$ ). Estimations of PUN, using SUN can be improved though by adding other effects to the model, such as Diet, Date and Seasonality. My results show that the relationship between PUN and SUN was moderately strong ( $R^2 = 0.65$ ) when using a simple linear model. Using this model, SUN can be estimated using PUN, and PUN can also be estimated using SUN. The reliability of these estimations is limited by the strength of the relationship. As there is only a moderately strong linear relationship between plasma and saliva urea N, this relationship equation cannot reliably predict PUN levels based on the saliva urea N levels. The improved model fit provided by the mixed model procedure increased the adjust  $R^2$  by 0.12 and reduced the root mean square error by 0.341 due to the inclusion of fixed effects such as Diet, Date and Seasonality. Therefore, evaluation of the diet and date of sampling can be used to improve reliability of the model to predict PUN from SUN concentration.

#### 4.1.2 Significant Effects on the relationship between saliva urea N and plasma urea N

The interaction between diet and sampling date had a significant effect on the relationship between SUN and PUN. Including the interaction effects of Month and Diet improved the  $R^2$  to  $\sim 0.75$  ( $P < 0.001$ ). This interaction is likely explained by the crude protein (CP) content of the diet. The CP content of the Diverse treatment was similar during April, June and July. The CP content of the PRG diet was lower in August than the CP content of the Diverse diet. However, In September the CP content of the diverse diet was less than the PRG treatment. These results are reflected in the SUN and PUN levels presented in Figure 3. While there was no dietary difference between both the SUN and PUN levels in June and July, the dietary differences observed in PUN and SUN in August and September appears to reflect the changes in dietary CP of the diets during these months. In August, animals in the PRG diet treatment had a lower SUN and PUN levels compared with animals in the Diverse diet treatment. In September, when the Diverse treatment had lower CP levels, animals also had lower SUN and PUN levels than those fed the PRG diet. This is consistent with the findings of Madsen (2022). Madsen, (2022) found the CP level within the diet is strongly correlated to urinary N

output of dairy cows. Crude protein levels in pastures change throughout the year, typically they increase over the warmer summer months and decline through the colder winter months (Malau-Aduli, 2007). Therefore, evaluation of the CP content of the animal's diet throughout the year can increase the reliability of PUN predictions when using SUN.

There was a significant effect of gender by seasonality interaction on SUN and PUN. However, the effect of this interaction is outside the scope of my study. Figure 3 presents both SUN and PUN levels in spikers, suggesting that, although PUN and SUN were numerically greater than that of hinds, they were statistically similar ( $P > 0.1$ ). The tendency of the interaction between gender and seasonality may support the natural effect of sexual dimorphism and in this case more marked between the high seasonality spikers and hinds. The effects of sexual dimorphism in red deer may allow for greater N use efficiency of the males in comparison to females (Wu, 2020). Yearling stags have a greater plane of nutrition and metabolic requirement than that of hinds. This means that they will typically use a comparatively greater proportion of N from the forage on maintenance and growth (Catchpole, 2004). Animals with greater N demand tend to utilise N much more efficiently. More efficient N utilisation allows for faster animal growth from a similar or lower N input and therefore, a potential reduction in negative environmental N output (Congreves, 2021), as N is diverted to tissue rather than excretion as urine or faeces. However, N intake was not measured in this study, therefore these speculations should be taken with caution. The interaction between gender and seasonality observed for PUN and SUN concentration requires further investigation.

#### **4.1.3 Estimation of plasma urea nitrogen from saliva urea nitrogen**

The main objective of the study was to determine whether an easy to measure animal variable like SUN could be used to as a tool to quickly evaluate a large number of deer for UN concentration estimates. Arguably, the handling of deer, especially mature animals, is not easy. Blood sampling, unless conducted by experienced personal, often requires 3-4 people and can create a stressful environment for the animal. Therefore, SUN sampling might emerge as a more practical alternative. The technique and methodology for collecting saliva samples for determining SUN in deer has not been well described in the literature and still needs further considerations and methodological refining. The exact anatomical location of the oral cavity, time required for sufficient swab saturation, and time of day are all factors which may impair the collection of a representative sample. The anatomy of the salivary glands in red deer differs to other domesticated ruminants as deer have (four glands? Or insert correct number here..) and each saliva gland may excrete saliva in different rates, with differing UN concentrations (reference). Secretion from the parotid gland contains a significantly greater urea concentration than secretion from both the submandibular gland and the sublingual gland (Kovalčíková, 2018). The parotid gland is the heaviest saliva gland in relation

to the animal's bodyweight and it is vital in ruminal digestion regulation (Kay, 1987). The variation in UN between saliva glands may explain some of the variation in the SUN concentrations of the present study and further evaluation of the methodology used to collect saliva samples is needed.

To improve the accuracy of predicting PUN by SUN -i.e., reducing SUN variability-, a more specific sampling site should be used, such as the saliva pocket within the right side of the jaw. This most likely will reduce between animal variation as well. The sampling method used in this trial could also be adapted more closely to the methods of trials looking at tannin-binding properties, such as the one by (Fickel, 1998). Fickel (1998) used collection catheters to collect the saliva from the parotid gland. Samples of mixed saliva were also collected and compared to parotid gland saliva for the reliability in relation to tannin-binding properties. Fickel (1998) also reports that using the saliva exclusive to the parotid gland presented an increased number of tannin-binding properties.

However, the use of collection catheters is more invasive than regular saliva sampling or blood sampling, as animals need to be euthanised before the catheter can be inserted are not practical for SUN determination (Lamy, 2012). Saliva samples are also often contaminated by ingesta and digesta and need to be carefully examined before SUN analysis (Lamy, 2012). A potential alternative to reduce contamination of digesta is to sample before their dawn grazing bout, as ruminants generally fast overnight. Contamination of saliva may also be reduced if sampling occurs during idling times, as residues of the rumination boli may be potentially less (Montgomery, 1963). However, individual animals often ruminate and idle during different periods throughout the day. Overall, sampling using mixed saliva is less stressful and safer, especially when handling larger stags, spikers, or hinds (Carragher, 1997). In order to reliably replace plasma with saliva sampling, further development to this model is required, using a larger sample size and investigating significant effects. With larger animals in greater number, this sampling technique still has the potential to become a reasonably non-invasive easy tool to quickly evaluate a large number of deer for UN concentration estimates (Bilancio, 2018; Kovalčíková, 2018; Lasisi, 2016; Raimann, 2016; Renda, 2017; Shannon, 1977).

## Chapter 5

### Conclusion

The implications of these findings are promising for the New Zealand deer industry. There is a significant linear relationship between SUN and PUN in red deer calves. Therefore, SUN can be used to estimate PUN and thereby, the potential difference in individual animal UN excretion can be calculated through saliva sampling. Using a simple linear regression, SUN explained 65% of the variation of PUN, therefore to increase the reliability of PUN estimates other factors/effects need to be considered in the predictive model, e.g., diet by sampling date as demonstrated in the present study. When the interaction between Month and Diet was included in the model ~75% of the variation of PUN was explained by SUN. Deer are not easy to handle, therefore, with careful sampling methods, SUN may emerge as an easy and practical sampling technique to evaluate large numbers of mature red deer.

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