The effect of sheep genetic merit and feed allowance on nitrogen partitioning and isotopic discrimination

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ABSTRACT

Animal nitrogen (**N**) partitioning is a key parameter for profitability and sustainability of ruminant production systems, which may be predicted from N isotopic discrimination or fractionation (Δ^{15}**N**). Both animal genetics and feeding level may interact and impact on N partitioning. Therefore, this study aimed to assess the interactive effects of genetic merit (**G**) and feed allowance (**F**) on N partitioning and Δ^{15}N in sheep. The sheep were drawn from two levels of G (high G vs. low G; based on New Zealand Sheep Improvement Limited (http://www.sil.co.nz/) dual (wool and meat) growth index) and allocated to two levels of F (1.7 (high F) vs. 1.1 (low F) times Metabolisable Energy requirement for maintenance) treatments. Twenty-four Coopworth rams were divided into four equal groups for a N balance study: high G × high F, high G × low F, low G × high F, and low G × low F. The main factors (G and F) and the interaction term were used for 2-way ANOVA and regression analysis. Higher F led to higher N excretions (urinary N (**UN**); faecal N (**FN**); manure N), retained N, N use efficiency (**NUE**), and urinary purine derivatives excretion ($P < 0.05$). On the other hand, higher UN/N intake, and plasma Δ^{15}N were observed with the lower F ($P < 0.05$). Higher G led to increased UN, FN, manure N, apparent N digestibility, and urinary purine derivatives excretion ($P < 0.05$). Higher F only increased UN in high G sheep, with no effect on low G sheep ($P < 0.05$). Regression analysis results demonstrated potential to use plasma Δ^{15}N to reflect the effects of G and F on NUE and UN/N intake. Further research is urged to study interactive effects of genetic and feeding level on sheep N partitioning.

Implications

This study showed the potential to use nitrogen isotopic discrimination as a biomarker to estimate urinary nitrogen excretion and nitrogen utilisation from sheep with divergent nitrogen intake. This approach could be used in future research testing management or breeding interventions designed to increase nitrogen utilisation by sheep.

Introduction

Adequate levels of dietary nitrogen (**N**) must be offered to animals in order to maximise production and profitability (Ferguson and Sklan, 2005). Studies showed that only 20–30% of dietary N is retained in animal products (e.g., milk and meat), while the rest is excreted in urine and faeces (Wessels and Titgemeyer, 1997; Doranalli et al., 2011). Urinary N excretion (**UN**) can reduce farm productivity, and it also has negative consequences for the environment through nitrate leaching and greenhouse gas (i.e., nitrous oxide) emissions. Further, when N is over supplied in the diet relative to requirement, the excess N is mainly excreted in the urine. Therefore, the UN/N intake is often used as a proxy to reflect N use inefficiency in animal production systems.

The standard method for estimating N partitioning in non-lactating animals is the N balance study, which requires separate collection of total urinary and faecal outputs for several days. However, this method is labour intensive and costly, especially when it is used with a large number of animals (MacRae et al., 1993; Spanghero and Kowalski, 1997). A recently developed method for evaluating N partitioning relies on the natural ^{15}N enrichment of animal proteins relative to feed (Cantalapiedra-Hijar et al., 2015).

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The natural abundance of ^{15}N ($\delta^{15}N$; $^{15}N/^{14}N$ portion relative to air N gas standard) in plasma is generally higher relative to the consumed feed (DeNiro and Epstein, 1981; Cheng et al., 2013a; 2013b). This is known as plasma N isotopic discrimination or fractionation (plasma $\Delta^{15}N$; plasma $\delta^{15}N$ – feed $\delta^{15}N$). Previous studies showed that the plasma $\Delta^{15}N$ can be an indicator to predict N use efficiency (**NUE**) of ruminants (Cheng et al., 2013a; 2013b; Cabrita et al., 2014; Cantalapiedra-Hijar et al., 2015). This was further evidenced by a recent meta-analysis, which included both large and small ruminants, growing and lactating ruminants offered a range of diets (Cantalapiedra-Hijar et al., 2018). Furthermore, earlier studies indicated the potential use of plasma $\Delta^{15}N$ to predict UN/N intake in different groups of ruminants such as non-lactating ewes (Cheng et al., 2013a; 2013b), as well as to predict UN in lambs offered different diets (Bernard et al., 2020).

It is well known that the genetics can affect N partitioning (Ferris et al., 1999; Cheng et al., 2015) and $\Delta^{15}N$ (Cheng et al., 2014). Genetic merit (**G**) interacts with environmental factors (e.g., feed allowance (**F**; defined as feed offered on the basis of metabolisable energy for maintenance per Cheng et al. (2015)) to determine phenotypic expression of production performance. However, to the best of our knowledge, no previous study has evaluated the interactive effects of G × environmental factor on N partitioning and $\Delta^{15}N$. Therefore, the present study aimed to investigate G × F interactive effects on both N partitioning and $\Delta^{15}N$ in sheep. The hypothesis for this study was that both G and F can affect N partitioning and $\Delta^{15}N$, resulting in negative and positive linear relationships between NUE and $\Delta^{15}N$, and UN/N intake and $\Delta^{15}N$, respectively. Moreover, we hypothesised that high G and low F groups can use N intake more efficiently than other groups.

Material and methods

Experimental design

This study was conducted as part of a project at Lincoln University, New Zealand (with approved Animal Ethics application number 536) to examine G × F interactive effects on nutrient utilisation of Coopworth sheep. The N balance study was conducted at the end of a live weight gain study (Cheng et al., 2015). Twenty-four Coopworth rams (aged: 11–12 months; weighing: 53 kg, s.d. = 7.8 kg) were allocated to four treatments (6 rams/treatment). The treatments consisted of two levels of G (low and high) and two levels of F (low and high). The high G and low G groups were selected using the New Zealand Sheep Improvement Limited (http://www.sil.co.nz/) dual (wool and meat) growth index. The G selection and control lines (i.e., low G) were established in 1986 for a long-term research study evaluating the rate of progress in G using single vs. multi-trait selection. The four groups were: high G × high F, high G × low F, low G × high F, and low G × low F. Each group was fed for six weeks – the first five in individual animal pens for a live weight gain study (Cheng et al., 2015), followed by a week in metabolic cages for this N balance study. Lucerne pellets were the only feed offered to sheep throughout the study, and all sheep had *ad libitum* access to water. The F was offered to provide 1.7 and 1.1 times of metabolisable energy for maintenance (MJ/day) for high F and low F groups, respectively, based on the Nicol and Brookes (2007) equation: metabolisable energy for maintenance = 0.5 × (live weight)$^{0.75}$.

Animal measurement

The first two days of the N balance study were used for adaptation to metabolic cages and the following five days (from day three to day seven) were used for measurement. The sheep were offered Lucerne pellets at 10.30 am daily according to the feed allowance calculation, based on the group average live weight at the beginning of measurement period (60.8 kg, 56.3 kg, 49.3 kg, and 45.2 kg, respectively, for high G × high F, high G × low F, low G × high F, and low G × low F groups). Daily feed samples were collected and bulked for chemical analysis. Daily urine and faeces were collected per sheep in the measurement period. A bucket with a layer of plastic mesh was placed under the drainage funnel of metabolism cage to allow urine to drain through the mesh, leaving faeces on top of the mesh for collection. Prior to urine collection, 250 ml of 10% (vol/vol) H_2SO_4 was added to each bucket to keep urine pH between 2 and 3. Blood samples were collected from the jugular vein into 10 ml Li-heparinised evacuated tubes during the last measurement day (2.30 pm) and plasma was obtained by centrifugation (15 minutes, 1 200*g* at 4 °C). All blood, plasma, urine and faeces samples were stored at −20 °C for further analysis.

Sample analytical methods

Blood, plasma, faeces, and feed samples were freeze-dried and analysed for $\delta^{15}N$ following the procedure described by Cheng et al. (2011) and isotope-ratio mass spectrometry (PDZ Europa Ltd, Crewe, UK). The equation: MJ metabolisable energy/kg DM = digestibility of organic DM (g/kg DM) × 0.016 was used to predict the dietary metabolisable energy content based on the study of Clarke et al. (1982). The N concentration in feed (freeze-dried), faeces (freeze-dried) and urine (thawed) were analysed using a Variomax CN analyzer (Elementar Analyzensysteme GmbH, Hanau, Germany). The HPLC (Agilent 1100 series, Waldbronn, Germany) was used to analyse urinary purine derivatives in urine, as described by George et al. (2006). To analyse plasma urea N (**PUN**) and plasma glucose, enzymatic kinetic methods were used on a Daytona RX Clinical Analyzer (Rondox, Nishonomiya, Japan). Retained N (**RN**; g/d), NUE (%), apparent N digestibility (%) were calculated using the following equations:

$$\text{RN (g/d)} = \text{N intake (g/d)} - \text{UN (g/d)} - \text{fecal N (\textbf{FN}) (g/d)}$$

$$\text{NUE (\%)} = \text{RN (g/d)} \div \text{N intake (g/d)} \times 100$$

$$\text{Apparent N digestibility (\%)} = [(\text{N intake (g/d)} - \text{FN (g/d)})] \div \text{N intake (g/d)} \times 100$$

Statistical analysis

GenStat (version 19; VSN International Ltd., Hemel Hempstead, UK) was used to perform an ANOVA (two-way). As sheep was the replication unit in this study, the pooled single values for each measured parameter per sheep were used for statistical analysis. The statistical model reported G, F, and G × F effects. The significant thresholds were set at P-value <0.05, and trends were declared at 0.05 < P-value < 0.10.

Results

Nitrogen partitioning

Higher F led to significantly higher N excretions (UN, FN, and manure N), RN, NUE, and urinary purine derivatives excretion than for the lower F (P < 0.05). On the other hand, a significant higher UN/N intake, and plasma $\Delta^{15}N$ were observed for the lower F (P < 0.05; Tables 1 and 2). Higher G increased UN, FN, manure N, and N digestibility significantly (P < 0.05; Table 1). Higher F only increased UN in high G sheep significantly, with no effect in the low G sheep (P = 0.046). Higher F increased FN and manure N

Table 1
Nitrogen partitioning in sheep of high and low breeding value fed diets based on Lucerne pellets at either high (1.7 times maintenance) or low (1.1 times maintenance) feed allowance.

Item	High G × high F	High G × low F	Low G × high F	Low G × low F		*P*-value		
					LSD[1]	G[2]	F[2]	G × F
Animal #	6	6	6	6				
Metabolisable energy intake (MJ/sheep/day)	16.6	10.9	14.1	9.1	–	–	–	–
DM intake, kg/sheep/day	1.64	1.08	1.39	0.91	0.002	<0.001	<0.001	<0.001
N intake, g/sheep/day	47.5	31.3	40.2	26.3	–	–	–	–
UN, g/sheep/day	19.2	14.0	13.0	12.7	3.39	0.004	0.023	0.046
FN, g/sheep/day	15.2	9.6	13.0	8.9	0.73	<0.001	<0.001	0.006
Manure N, g/sheep/day	34.4	23.6	26.0	21.6	3.34	<0.001	<0.001	0.010
RN, g/sheep/day	13.1	7.7	14.2	4.7	3.32	0.125	<0.001	0.217
UN/N intake, g/g	0.40	0.45	0.32	0.48	0.097	0.488	<0.007	0.095
DM digestibility, %	59.0	59.5	58.8	55.9	1.90	0.010	0.075	0.017
N digestibility, %	69.1	69.8	67.8	66.3	1.89	<0.001	0.496	0.110
NUE[3], %	27.6	24.6	35.3	18.0	9.60	0.886	0.006	0.039
Urinary purine derivatives, mmol/day	17.5	8.4	12.2	8.7	2.71	0.013	<0.001	0.006

Abbreviations: High G × high F = high genetic merit and high feed allowance; High G × low F = high genetic merit and low feed allowance; Low G × high F = low genetic merit and high feed allowance; Low G × low F = low genetic merit and low feed allowance; UN = urinary nitrogen; FN = faecal nitrogen; RN = retained nitrogen.
[1] LSD = the least significant differences of means for G × F interaction.
[2] G = genetic merit; F = feed allowance.
[3] Nitrogen use efficiency = 100 × (RN/N intake).

Table 2
Plasma urea nitrogen, plasma glucose and natural enrichment of nitrogen-15 in feed, blood, and plasma, and nitrogen isotopic discrimination ($\Delta^{15}N$) of high vs. low genetic merit sheep offered two different feed allowances.

Item	High G × high F	High G × low F	Low G × high F	Low G × low F		*P*-value		
					LSD[1]	G[2]	F[2]	G × F
PUN, mmol/L	7.7	7.1	8.0	7.0	1.12	0.81	0.05	0.52
Plasma glucose, mmol/L	3.7	3.8	3.7	3.4	0.38	0.09	0.47	0.24
Feed $\delta^{15}N$, ‰	0.87	0.84	0.77	0.82	–	–	–	–
Blood $\delta^{15}N$, ‰	5.6	6.2	5.6	5.9	0.24	0.09	<0.001	0.23
Plasma $\delta^{15}N$, ‰	5.0	5.7	5.1	5.5	0.24	0.53	<0.001	0.21
Blood $\Delta^{15}N$, ‰	4.8	5.3	4.7	5.1	0.24	0.09	<0.001	0.23
Plasma $\Delta^{15}N$, ‰	4.2	4.8	4.3	4.7	0.24	0.53	<0.001	0.21

Abbreviations: High G × high F = high genetic merit and high feed allowance; High G × low F = high genetic merit and low feed allowance; Low G × high F = low genetic merit and high feed allowance; Low G × low F = low genetic merit and low feed allowance; PUN = plasma urea nitrogen.
[1] LSD = the least significant differences of means for G × F interaction.
[2] G = genetic merit; F = feed allowance.

significantly in both high G and low G sheep ($P = 0.006$ and $P = 0.01$ for interaction effects, respectively; Table 1). The NUE was affected significantly ($P = 0.039$) by F only in low G sheep, with no effect in high G sheep. The NUE for high G × high F, high G × low F, low G × high F, and low G × low F were 28, 25, 35, and 18%, respectively. The high G × high F group excreted the most UN (19.2 g/d; $P < 0.05$).

Nitrogen isotopic discrimination

There was a strong positive linear relationship between blood $\Delta^{15}N$ and plasma $\Delta^{15}N$ (Fig. 1). Lower plasma and blood $\Delta^{15}N$ ($P < 0.001$; Table 2) were found in low vs. high F irrespective of G (G × F interaction; $P \geq 0.21$). Conversely, higher PUN concentrations were observed in low vs. high F also regardless of G (G × F interaction; $P = 0.52$). There was no significant effect of G on any of these parameters ($P > 0.05$).

Discussion

The current study was conducted as part of research (Cheng et al., 2015) at Lincoln University, to examine G × F interactive effects on nutrient utilisation of Coopworth sheep. Cheng et al. (2015) found no effect of G on live weight gain, feed conversion efficiency, PUN, plasma glucose, blood and plasma $\Delta^{15}N$. Moreover, the high F group had higher live weight gain and feed conversion efficiency, and in all cases, the low F group had a higher blood and plasma $\Delta^{15}N$ than the high F group.

Nitrogen metabolism and partitioning

The difference in DM intake can be related to G (regardless of sheep initial live weight), as the selection of high growth rate sheep is usually associated with a higher intake/feed demand (Coleman et al., 2010; Cheng et al., 2014). In the study by Cheng et al. (2014), cows with high compared to low breeding worth (a composite genetic index including milk production) had a higher N intake (and NUE), but a lower UN. However, in the present study, N intake was higher for high G than low G, which led to more UN. The highest UN was from the high G × high F group, presumably since NI for the high G × high F group was 52%, 18%, and 81% higher than high G × low F, low G × high F, and low G × low F groups, respectively. The FN increased as DM intake increased, which is similar to observations in dairy cows (Moorby et al., 2006; Niu et al., 2016); and the increased FN was also due to increased N intake. This is consistent with the other reports (Niu et al., 2016; Kidane et al., 2018), which clearly indicated that as animals consumed more N, they excreted more FN.

The UN/N intake ranged between 0.3 and 0.5 g/g in this study, which was lower than that reported by Brand et al. (1992). A low

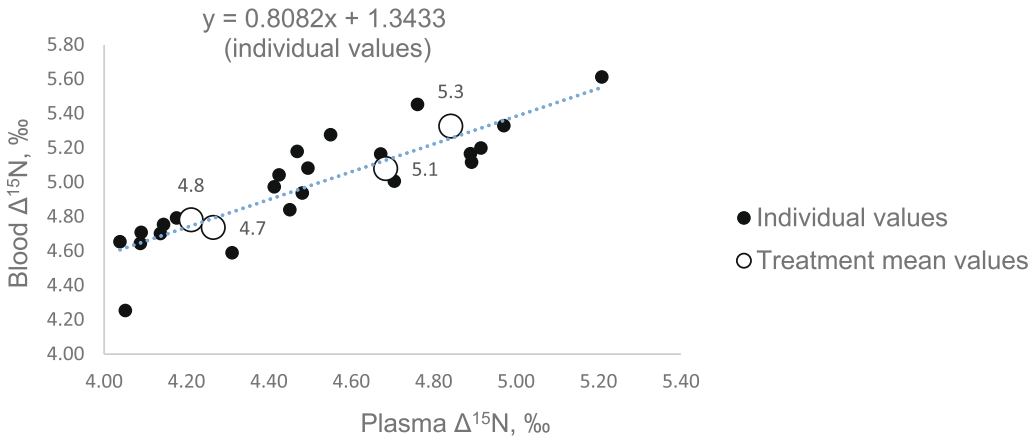

Fig. 1. The relationship between blood nitrogen isotopic discrimination (blood $\Delta^{15}N$) and plasma $\Delta^{15}N$ for individual observations and treatment mean values (labelled) of high vs. low genetic merit sheep offered two different feed allowances.

F led to increased UN/N intake and a lower NUE in these sheep compared with their counterparts offered with high F. The reason behind this is unclear. It is important to note that yearling sheep were used in the current study, in contrast to the adult sheep used in the study by Brand et al. (1992). It is known that increased UN/N intake occurs as animal ages; less protein is retained with declining

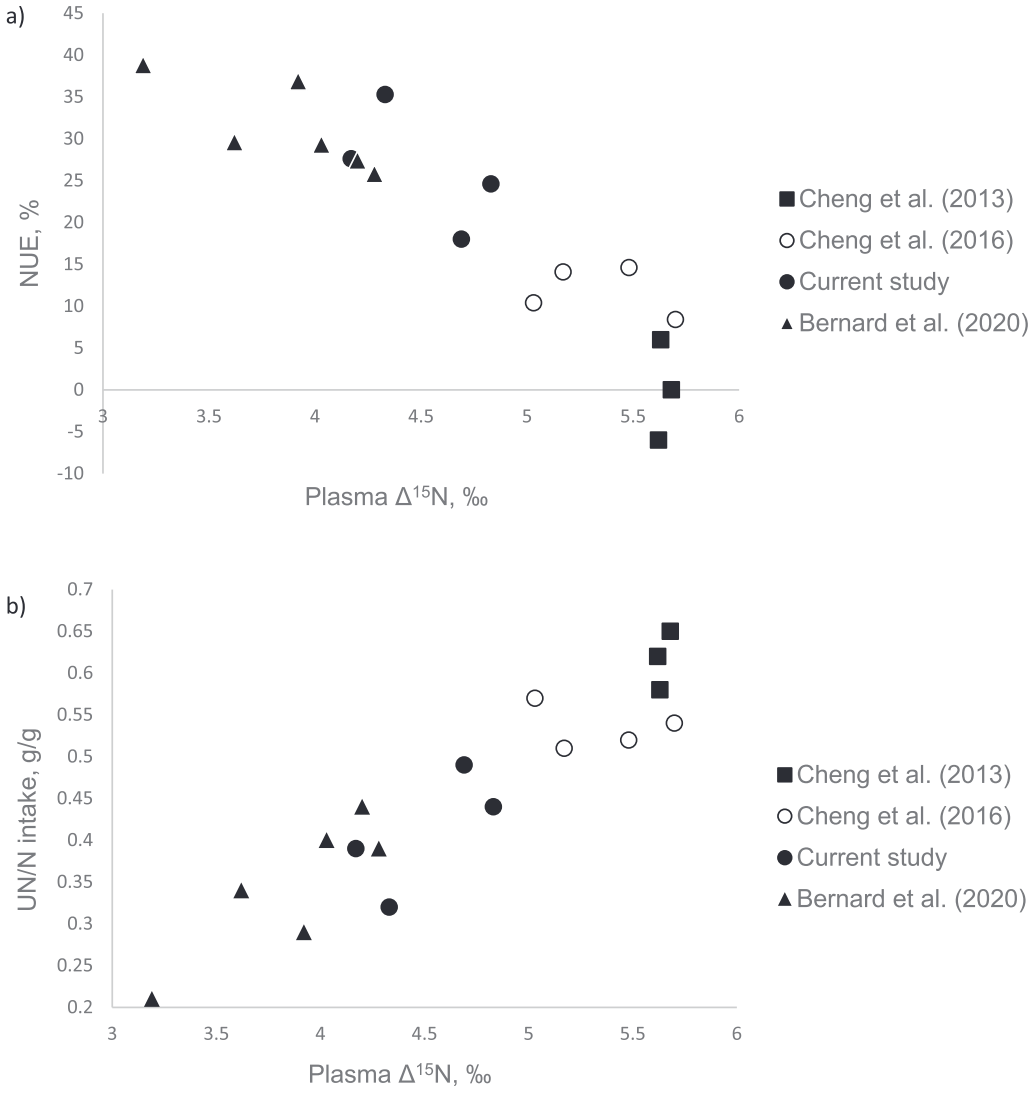

Fig. 2. The relationships between nitrogen use efficiency (NUE) or urinary nitrogen to nitrogen intake ratio (UN/N intake) and with plasma nitrogen isotopic discrimination (plasma $\Delta^{15}N$) for treatment mean values from literature review: Cheng et al., 2013a and 2013b (in non-lactating sheep); Cheng et al., 2016 (in lactating goats); Bernard et al., 2020 (in growing lamb) and the current study (in non-growing rams): (a) NUE vs. plasma $\Delta^{15}N$; (b) UN/N intake vs. plasma $\Delta^{15}N$.

metabolisable protein requirements (INRA, 2018). The effects of G on N digestibility (i.e., higher for high G groups than low G groups) are not consistent with the results from a study by Ferris et al. (1999) in dairy cows. The urinary purine derivatives result for high F groups supported this result; it seems likely that higher rumen microbial protein synthesis leads to a higher RN and NUE (Dewhurst et al., 1996; Cheng et al., 2011).

Nitrogen isotopic discrimination and plasma urea nitrogen

The PUN is a widely used biomarker to evaluate N partitioning (Kohn et al., 2005). In the current study, PUN concentrations reflected well the difference in N intake across treatments and were within the range described by Kohn et al. (2005) and Cheng et al. (2013a and 2013b). The range of plasma $\Delta^{15}N$ values in this study is comparable with the range reported by previous studies in ruminants (Cheng et al., 2013a; 2013b; Wheadon et al., 2013; Cantalapiedra-Hijar et al., 2015). A positive relationship between plasma $\delta^{15}N$ and blood $\delta^{15}N$ is expected (Cheng et al., 2015). Plasma was less enriched in ^{15}N than blood, which can be because of the different turnover rate of plasma protein in comparison with red blood cells (Sick et al., 1997; Cheng et al., 2010; 2015). Blood $\Delta^{15}N$ and plasma $\Delta^{15}N$ were very similarly ranked across the four treatments, which suggests that there was a long enough adaptation period in this study (i.e., this study was conducted at the end of the 47-day live weight gain study reported by Cheng et al. (2015)).

Nitrogen utilisation in relation to nitrogen isotopic discrimination

The more efficiently the animals use dietary N, the lower the plasma $\Delta^{15}N$ (Sick et al., 1997; Cheng et al., 2013a; 2013b). The NUE increased when F and N intake increased in this study, which contrasts with the result of some previous studies (e.g., a cow study from Cheng et al. (2011); and a sheep study from Bernard et al. (2020)). Spanghero and Kowalski (1997) indicated that as N intake increased, the errors (overestimation) of N balance increased. The reason for this is not clear, but it may in part be attributed to the different experimental conditions between studies, including species differences and as previously discussed, sheep age. Additionally, in the current study, increased F affected ratio of metabolisable energy and metabolisable protein (perhaps partly through increased rumen microbial protein synthesis), which affected the ability of the growing animal to retain N. Further in this study, G did not affect NUE. This is not consistent with other studies in dairy cows (Ferris et al., 1999; Wheadon et al., 2013), which showed that high G cows used N more efficiently than medium and low G cows. Nevertheless, there is limited sheep G × F literature on N balance and more research is needed. A negative relationship between NUE and plasma $\Delta^{15}N$ was found in the current study, which is consistent with the other sheep studies (Cheng et al., 2013a, 2013b; 2016; Bernard et al., 2020; Fig. 2a). This negative relationship may be related more to N metabolism than N digestion in the animal, which contributes to NUE and plasma $\Delta^{15}N$ changes (Cantalapiedra-Hijar et al., 2018).

Urinary nitrogen to nitrogen intake ratio in relation to nitrogen isotopic discrimination

Plotting the treatment mean values from the current study with sheep data from the literature (Cheng et al., 2013a, 2013b; 2016; Bernard et al., 2020; Fig. 2b) showed a positive linear relationship between UN/N intake and plasma $\Delta^{15}N$. The underlining reason for this relationship is possibly related to a common contribution of N metabolism (namely deamination and transamination) to N partitioning and discrimination (Cantalapiedra-Hijar et al., 2018). Additionally, the relationship between the N digestibility and plasma $\Delta^{15}N$ (r^2 = 0.01) was low, suggesting that digestion contributes little to the overall relationship between UN/N intake and $\Delta^{15}N$. It is interesting to note that this study showed that plasma $\Delta^{15}N$ was not able to discern effects of G on UN/N intake; however, it was good enough to differentiate effects of F on UN/N intake. This may be because F had significant effects on plasma $\Delta^{15}N$ and UN/N intake, while G did not affect plasma $\Delta^{15}N$ and UN/N intake. Further studies should explore these relationships with both growing and mature sheep.

Conclusion

A low F led to increased UN/N intake and a lower NUE in these sheep. Plotting treatment mean values from the current study alongside literature data showed positive and negative linear relationships between UN/N intake and plasma $\Delta^{15}N$, and NUE and plasma $\Delta^{15}N$, respectively. These results demonstrate the potential use of plasma $\Delta^{15}N$ to reflect NUE and UN/N intake when G and F interactive effects are considered. However, more studies are needed to explore genotype and diet interactive effects on N metabolism.

Ethics approval

These study procedures were performed and approved in accordance with the guidelines of Lincoln University Animal Ethics Committee.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

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Declaration of interest

The authors declare no conflict of interest.

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