

Effects of feed intake and genetics on tissue nitrogen-15 enrichment and feed conversion efficiency in sheep¹

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ABSTRACT: This study investigated the effects of sheep genetics and feed intake on nitrogen isotopic fractionation ($\Delta^{15}\text{N}$) and feed conversion efficiency (FCE; live weight gain/DMI), using a 2×2 factorial design, with 2 levels of genetic merit for growth (high vs. low) and 2 levels of feed intake (110 vs. 170% of ME for maintenance [MEM]). No effect of genetic merit was detected for live weight gain ($P = 0.64$), FCE ($P = 0.46$), plasma urea nitrogen ($P = 0.52$), plasma glucose ($P = 0.78$), and $\Delta^{15}\text{N}$ of wool ($P = 0.45$), blood ($P = 0.09$), and plasma ($P = 0.51$). Sheep receiving 170% of MEM had 175% higher live weight gain ($P < 0.001$) and 77% higher FCE ($P < 0.001$) than sheep receiving 110% of

MEM. There was no difference among treatments at the beginning of the study for either blood or plasma $\Delta^{15}\text{N}$, but the treatment groups started to diverge in blood and plasma $\Delta^{15}\text{N}$ at 21 and 7 d, respectively. Blood, plasma, and wool samples were enriched in ^{15}N compared with feed. There was a higher blood, plasma, and wool $\Delta^{15}\text{N}$ for the low feed intake group than the high feed intake group ($P < 0.001$ in all cases). Across the 4 treatment groups, higher FCE in sheep was associated with lower $\Delta^{15}\text{N}$ for plasma, blood, and wool. Overall, the results are consistent with the potential of $\Delta^{15}\text{N}$ as a rapid, low-cost biomarker of FCE in sheep, despite there being no effects of genetic treatment on FCE and $\Delta^{15}\text{N}$.

Key words: genetic and nutritional interaction, growth potential, isotopic discrimination, live weight gain, nitrogen-15

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INTRODUCTION

Breeding for increased animal feed conversion efficiency (FCE; live weight [LW] gain/DMI) is important for a sustainable livestock industry. However, the substantial genetic variation for FCE in cattle and sheep (Berry and Crowley, 2013) has not been exploited because of the difficulty and cost of recording feed intake and LW changes (Herd et al., 2003). Candidate metabolites or hormone biomarkers have

been explored but did not provide consistent relationships with FCE (Kelly et al., 2010; Lawrence et al., 2012). Recently, Wheadon et al. (2014) found a significant negative correlation between FCE and plasma N isotopic fractionation ($\Delta^{15}\text{N}$; animal $\delta^{15}\text{N}$ – feed $\delta^{15}\text{N}$) in beef cattle, and similar correlations were also reported in earlier work by Cheng et al. (2013a,b), which showed that N use efficiency (product N/N intake) in sheep and dairy cows was negatively related to $\Delta^{15}\text{N}$ of muscle and plasma, respectively. Previous work showed the change in $\Delta^{15}\text{N}$ was related to major N metabolism reactions such as liver transamination (Macko et al., 1986) and rumen microbial protein synthesis (Wattiaux and Reed, 1995). Although there is tentative evidence for an association between FCE and $\Delta^{15}\text{N}$ from comparisons of physiological states and effects of dietary treatments that are expected to alter FCE (Sponheimer et al., 2003), it is only recently that the association was confirmed using measurements of FCE for beef cattle offered identical diets (Wheadon et al., 2014). It is not clear if

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Table 1. Average chemical composition (mean and SD) of feed samples collected over the 5-wk measurement period ($n = 20$)

Item	Feed composition	
	Mean	SD
DM, g/kg	888	5.5
Fat, g/kg DM	24	1.0
NDF, g/kg DM	458	16.0
Digestible OM in DM, g/kg DM	607	15.4
CP, g/kg DM	188	6.5
ME, MJ/kg DM	9.7	0.30

such an association would also be observed with other species and also when considering genetic differences between groups of animals and when different diets were offered to animals. The objective of this study was to evaluate sheep genetic and feed intake effects on $\Delta^{15}\text{N}$ and FCE and the association between $\Delta^{15}\text{N}$ and FCE and to compare 3 types of samples (blood, plasma, and wool) for determination of $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$.

MATERIALS AND METHODS

Experimental Design

Twenty-four 11- to 12-mo-old Coopworth rams were used in this study. Two levels of genetic merit and 2 levels of feed intake were evaluated using a 2×2 factorial design. The 4 treatments were high genetic merit + high feed intake (**HGHF**), high genetic merit + low feed intake (**HGLF**), low genetic merit + high feed intake (**LGHF**), and low genetic merit + low feed intake (**LGLF**). Each treatment group had 6 sheep and they were fed for 1 wk as diet adaptation and 5 wk for LW measurement and sample collection.

Animal Selection, Diet, and Feed Allowance

The Coopworth flock from Ashley Dene Research Farm, Lincoln University, Lincoln, New Zealand, was used to supply sheep for this study. The high genetic merit (**HG**) and low genetic merit (**LG**) groups were selected based on the New Zealand Sheep Improvement Limited (<http://www.sil.co.nz/>) dual purpose for growth. The selection line and control line were established in 1986 to support a long-term research project investigating the rate of genetic progress using single trait or multitrait selection. The mean (SD) were 1,677 (158.9) New Zealand cents (¢) and 180 (185.6) New Zealand ¢ for HG and LG, respectively. Each group was then divided into 2 subgroups for high feed intake (**HF**) and low feed intake (**LF**), balanced for LW measured at the start of the measurement period (Table 1).

All sheep grazed on ryegrass and clover based pasture before the study. Alfalfa pellets (Seales Winslow Ltd., Ashburton, New Zealand) were gradually introduced to the sheep for 1 wk before the study commenced. Two days before the measurement period, sheep were consuming a diet of 100% alfalfa pellet. Throughout the study, fresh water was offered ad libitum. The feeding allowance was designed to provide either 110% of ME for maintenance (**MEM**) for LF groups or 170% of MEM for HF groups, based on the equation $\text{MEM} = 0.5 \times \text{LW}^{0.75}$ (Nicol and Brookes, 2007).

Animal Measurements

The study was undertaken under the authority of Lincoln University Animal Ethics Committee (application number 536). Sheep were individually housed in pens with feed offered once a day at 1030 h. All sheep were weighed (XR 3000; Tru-Test Group, Auckland, New Zealand) after 12 h overnight fasting at the start and the end of the 5 wk measurement period. Each sheep was offered alfalfa pellets daily, based on calculated feed allowance according to group average LW measured at the start of the measurement period. Feed refusals were recorded to calculate DMI. In addition, FCE was calculated using the equation $\text{FCE (g/kg)} = \text{LW gain (g/d)}/\text{DMI (kg/d)}$. Weekly feed samples from each treatment group were collected and stored at -20°C before compositional and $\delta^{15}\text{N}$ analysis.

Blood samples were collected from the jugular vein of each sheep into 10 mL Li-heparinized evacuated tubes at 1430 h on the first day of measurement Week 1 and at the last day of each measurement week. Blood was subsampled into tubes to harvest plasma by centrifugation at $1,200 \times g$ for 15 min at 4°C and then both blood and plasma were stored at -20°C . A total of 120 blood samples were obtained from this study. There were 9 hemolyzed samples and the corresponding plasma samples were excluded for $\delta^{15}\text{N}$ and glucose analysis due to contamination of plasma with red blood cells after centrifugation. A 5-mm wool sample per sheep was shaved from skin level 2 wk after the completion of the study (Rogers and Schlink, 2010) for $\delta^{15}\text{N}$ analysis.

Chemical Analysis

Alfalfa pellet samples were bulked per treatment at the end of the study and then ground to pass through a 1.0-mm sieve (Christy Lab Mill, Suffolk, UK). The fat, CP, digestible OM in DM (**DOMD**), and NDF were predicted by near-infrared spectroscopy (Feed and Forage Foss Analyzer 500; FOSS Analytical, Hillerod, Denmark), which is regularly calibrated with results from wet chemistry. The estimated ME content was

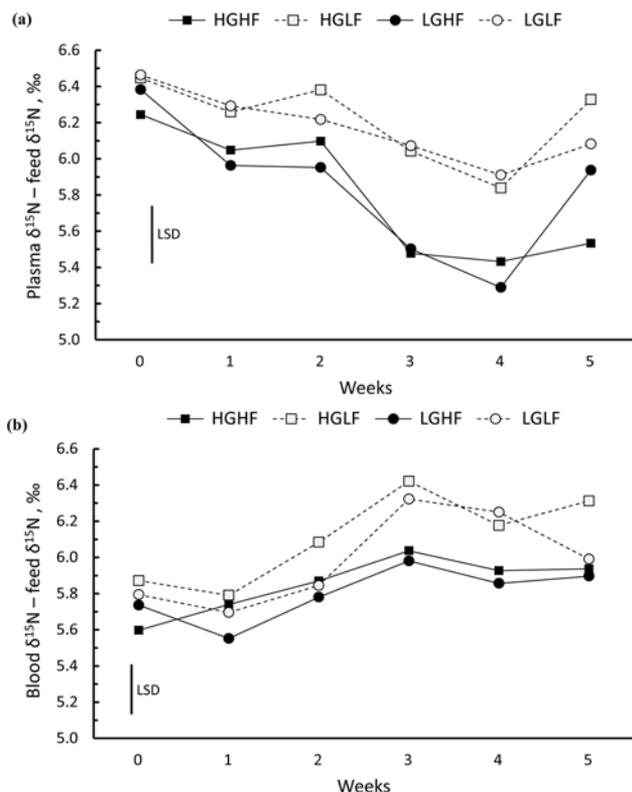


Figure 1. The change of N isotopic fractionation in plasma (1a) and blood (1b) over 5 wk of measurement period for high (H) and low (L) genetic merit sheep offered high and low feed intake (F). LSD, $P < 0.05$. HGHF = high genetic merit + high feed intake; HGLF = high genetic merit + low feed intake; LGHF = low genetic merit + high feed intake; LGLF = low genetic merit + low feed intake.

calculated using the formula from Clarke et al. (1982): $\text{ME (MJ/kg DM)} = \text{DOMD (g/kg)} \times 0.016$. Feed, blood, plasma, and wool samples were freeze-dried and analyzed for $\delta^{15}\text{N}$ according to the method described by Cheng et al. (2011) using an isotope-ratio mass spectrometer (PDZ Europa Ltd., Crewe, UK). Plasma urea nitrogen and glucose were analyzed by using an enzymatic kinetic method on a Daytona RX Clinical Analyzer (Randox, Nishonomiya, Japan).

Statistical Analysis

All statistical analyses were performed using Genstat version 16 (VSN International Ltd., Hemel Hempstead, UK). Results were considered statistically significant at $P < 0.05$.

For LW (at Weeks 0 and 5), LW gain, and FCE (over the whole 5 wk experimental period), the effects of feed intake and genetic merit and the interaction between these 2 main factors were assessed by 2-way ANOVA.

Plasma urea N and glucose data were also analyzed using 2-way ANOVA, with main factors feed intake and genetic merit and the interaction term, based on the mean values for each sheep obtained over the 5-wk experimen-

tal period. Wool $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ data were analyzed by similar 2-way ANOVA based on values obtained from the single measurement of wool $\delta^{15}\text{N}$ for each sheep.

For blood $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ and plasma $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$, an antedependence analysis was performed to determine at which weeks from the start of the experiment these variables became independent of preceding values (thus avoiding difficulties related to autocorrelation of data between time points). This antedependence assessment resulted in the data for blood $\Delta^{15}\text{N}$ from Weeks 4 through 5 and for plasma $\Delta^{15}\text{N}$ from Weeks 2 through 5 being considered independent from prior values (see Fig. 1). Therefore, to assess the effects of feed intake and genetic merit on these variables, 2-way ANOVA was performed on the average values for each sheep from Weeks 4 through 5 for blood and Weeks 2 through 5 for plasma.

The way in which the responses of blood $\Delta^{15}\text{N}$ and plasma $\Delta^{15}\text{N}$ to feed intake and genetic merit were affected by time from commencement of the different diet regimes were further analyzed using repeated measures ANOVA. For this analysis, any missing values for individual sheep at specific time points were replaced using maximum likelihood estimates.

To examine the relationships between plasma $\Delta^{15}\text{N}$, blood $\Delta^{15}\text{N}$, wool $\Delta^{15}\text{N}$, and FCE, Pearson's correlation coefficient was calculated using the 4 group means.

RESULTS

The average chemical composition of the alfalfa pellets is shown in Table 1; negligible variation was found over time and between treatment groups for all the components measured.

The HG animals were heavier than the LG animals at both Week 0 and Week 5 ($P < 0.001$; Table 2). Although there was no difference between feed intake groups for LW at the beginning of the study ($P = 0.65$; Week 0), the HF animals were heavier than LF at the end of the study (Week 5; $P = 0.02$; Table 2). The HF animals had 175% higher LW gain ($P < 0.001$) and 77% higher FCE ($P < 0.001$) than the LF animals (Table 2).

The HF animals had higher plasma glucose concentrations than the LF animals ($P = 0.01$; Table 3). Blood, plasma, and wool samples measured for $\delta^{15}\text{N}$ in this study were enriched in ¹⁵N compared with feed. There was significantly higher $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ for blood, plasma, and wool in the LF groups than in the HF groups ($P < 0.001$; Table 3).

There were statistically significant interactions between genetic merit, feed intake, and time points for blood $\Delta^{15}\text{N}$ ($F_{5, 100} = 2.90, P = 0.04$) and plasma $\Delta^{15}\text{N}$ ($F_{5, 100} = 7.09, P < 0.001$) over the 5-wk study (Fig. 1). The general trend was that plasma $\Delta^{15}\text{N}$ decreased over

Table 2. Dual purpose for growth (DPG),¹ DMI, live weight (LW) gain, and feed conversion efficiency (FCE) high and low genetic merit sheep offered high and low feed intake (mean; $n = 6$)

Item	HGHF ²	HGLF ²	LGHF ²	LGLF ²	LSD ³	P-value		
						Genetic merit	Feed intake	Genetic merit × feed intake
DPG, cents (¢)	1,666	1,690	142	218	–	–	–	–
DMI, kg/d	1.7	1.1	1.5	1.0	–	–	–	–
LW at Week 0, kg	52.6	52.0	43.4	42.2	5.83	***	NS ⁴	NS
LW at Week 5, kg	58.7	53.8	48.6	44.4	5.40	***	*	NS
LW gain, g/d	173.8	52.4	147.6	64.3	44.37	NS	***	NS
FCE, ⁵ g/kg	102.6	47.6	100.6	67.3	35.13	NS	***	NS

¹DPG is calculated genetic potential for LW gain by the New Zealand Sheep Improvement Limited (<http://www.sil.co.nz/>).

²HGHF = high genetic merit + high feed intake; HGLF = high genetic merit + low feed intake; LGHF = low genetic merit + high feed intake; LGLF = low genetic merit + low feed intake.

³ $P < 0.05$.

⁴NS = non-significant.

⁵FCE = LW gain/DMI.

* $P < 0.05$; *** $P < 0.001$.

the course of the study (Fig. 1a) whereas blood $\Delta^{15}\text{N}$ tended to increase (Fig. 1b). For plasma $\Delta^{15}\text{N}$, there was as strong interaction between time and feed intake treatment (F_5 , $100 = 8.62$, $P < 0.001$), with the feed intake groups starting to diverge after just 1 wk, with the LF group having a higher $\Delta^{15}\text{N}$ than the HF group (Fig. 1a). A similar significant divergence occurred in the blood $\Delta^{15}\text{N}$ (F_5 , $100 = 4.43$, $P < 0.006$), with the LF group having a higher $\Delta^{15}\text{N}$ than the HF group, although this time the separation was not so distinct and did not become apparent until Week 3.

The $\Delta^{15}\text{N}$ for plasma, blood, and wool all responded in a similar way among the 4 treatment groups of sheep, and therefore, significant positive relationships were observed between these variables (Table 4). Conversely, FCE was negatively related to all 3 $\Delta^{15}\text{N}$ measurements (Table 4).

DISCUSSION

Animal Performance

Similar trends across the 4 treatment groups were found for LW gain and FCE, with HGHF having the highest values followed by LGHF, LGLF, and HGLF. The higher LW gain and FCE observed for HF animals compared with LF animals is consistent with previous sheep studies (Cottle, 2003; El-Sabagh et al., 2013), and it can be explained by the well-established positive association between DMI and LW gain (Nicol and Brookes, 2007). The higher plasma glucose concentration found in HF animals than in LF animals suggests that more energy is available for rumen fermentation and nutrient absorption of sheep offered HF compared with LF. On the other hand, the higher feeding level may lead to lower MEm cost relative to ME available

for LW gain. Such “dilution of maintenance cost” effects allow higher proportions of ME to be used for production, leading to higher LW gain and FCE.

Although genetic effect was not observed for LW gain and FCE, it is interesting to note that LW gain and FCE were 23 and 14% higher for LGLF than for HGLF, respectively. This may be related to the different growth potential of LG and HG sheep under restricted feed intake, which relates to the requirement of MEm. The use of $\text{MEm} = 0.5 \times \text{LW}^{0.75}$ (Nicol and Brookes, 2007) as a general MEm prediction equation may not be suited for sheep, which differ in growth potential. Previous research suggested that high-producing dairy cows had larger internal organs than low-producing dairy cows (Ferrell and Jenkins, 1984), which can lead to a higher MEm requirement. This is supported by a recent trial in beef cattle, which showed higher FCE (measured as lower residual feed intake) due to a lower weight of the reticulorumen (Fitzsimons et al., 2014). Therefore, different MEm prediction equations may be needed to differentiate genetic differences in maintenance cost of animals.

Feed Conversion Efficiency in Relation to Nitrogen Isotopic Fractionation

A feature of the results from this study was the strong negative association between the group mean $\Delta^{15}\text{N}$ values and FCE. Earlier studies have shown weak associations between FCE and single blood metabolite or hormone measurements. For example, Kelly et al. (2010) reported weak correlations between FCE and plasma leptin ($r^2 = 0.23$), urea ($r^2 = 0.18$), and NEFA ($r^2 = 0.10$) in heifers, and Richardson et al. (2004) found only weak correlations between FCE and plasma glucose ($r^2 = 0.21$), aspartate aminotransferase ($r^2 = 0.20$), and albumin ($r^2 = 0.23$) in steers. Nitrogen isotopic

Table 3. Plasma urea N, plasma glucose, and N-15 concentrations (delta units expressed relative to standard air; ‰) in the feed, blood, plasma, and wool of high and low genetic merit sheep offered high and low feed intake (mean; *n* = 6)

Item	HGHF ¹	HGLF ¹	LGHF ¹	LGLF ¹	LSD ²	P-value		
						Genetic merit	Feed intake	Genetic merit × feed intake
Plasma urea N, mmol/L ³	8.5	7.4	8.0	8.4	1.06	NS ⁴	NS	NS
Plasma glucose, mmol/L ³	3.8	3.7	4.0	3.6	0.30	NS	*	NS
Feed δ ¹⁵ N, ‰	0.09	0.17	0.16	0.08	—	—	—	—
Blood δ ¹⁵ N, ‰ ⁵	5.91	6.33	5.87	6.06	0.231	NS	***	NS
Plasma δ ¹⁵ N, ‰ ⁶	5.66	6.24	5.74	6.02	0.273	NS	***	NS
Wool δ ¹⁵ N, ‰ ⁷	5.10	6.42	5.26	5.98	0.492	NS	***	NS
Blood Δ ¹⁵ N, ‰ ⁵	5.82	6.16	5.71	5.99	0.231	NS	***	NS
Plasma Δ ¹⁵ N, ‰ ⁶	5.57	6.07	5.57	5.94	0.273	NS	***	NS
Wool Δ ¹⁵ N, ‰ ⁷	5.01	6.25	5.10	5.90	0.492	NS	***	NS

¹HGHF = high genetic merit + high feed intake; HGLF = high genetic merit + low feed intake; LGHF = low genetic merit + high feed intake; LGLF = low genetic merit + low feed intake.

²*P* < 0.05.

³Based on a mean value for each sheep over the 5 wk of the experiment.

⁴NS = non-significant.

⁵Based on a mean value for each sheep from Weeks 4 through 5.

⁶Based on a mean value for each sheep from Weeks 2 through 5.

⁷Based on a single value for each sheep taken 2 wk after completion of the study.

P* < 0.05; **P* < 0.001.

fractionation provided a good indication of FCE in this study, and it seems likely that this association was driven by the partitioning of N between LW gain and excretion in urine. The negative association between Δ¹⁵N and FCE is consistent with the increased Δ¹⁵N when cattle and goats were fed diets containing higher protein levels (Sponheimer et al., 2003). It is also supported by the findings of Wheadon et al. (2014), who showed a strong negative relationship between individual cattle FCE and plasma Δ¹⁵N when they were offered the same diet.

Nitrogen Isotopic Fractionation

The enrichment of wool, plasma, and blood in ¹⁵N compared with feed is consistent with the literature (Koyama, 1985; Sponheimer et al., 2003). The main reason for the enrichment of ¹⁵N in these samples was probably due to the major N components being true protein or AA, which are naturally enriched in ¹⁵N (Sick et al., 1997; Cheng et al., 2010). The average levels of plasma Δ¹⁵N in this study was 5.66 ‰, which is in the upper end of the range previously reported for both lactating and nonlactating ruminants (Cheng et al., 2013b; Wheadon et al., 2014). The reason for this high plasma δ¹⁵N value in the current study is not clear, but it may be related to the maturity of the animals. Despite the high LW gain observed from the sheep used in this study, the sheep were close to maturity (Cottle, 2003), so an increased level of fat deposition might contribute to LW gain. Higher Δ¹⁵N in tissues should be expected from these sheep compared with younger individuals because mature sheep utilize feed protein less efficiently than suckling or lactating ani-

mals (Wheadon et al., 2014). For this reason, the stage of maturity of animals should also be considered when discussing isotopic fractionation in animal tissues.

A strong positive relationship was observed between Δ¹⁵N in plasma and blood, and this is expected as plasma is a fraction of blood. Blood was relatively more enriched in ¹⁵N than plasma in this study. This is because red blood cells in the blood contain mostly true protein, which is enriched in ¹⁵N relative to the urea containing plasma (Sick et al., 1997). Furthermore, it may be related to differences in the AA profile of free and protein-bound AA in plasma and red blood cells. For example, serine is a major AA in red blood cells but is not found in plasma (Canepa et al., 2002). Serine had a high isotopic fractionation factor among the 17 AA analyzed by Zhang and Altabet (2008). The positive relationship between plasma and wool Δ¹⁵N in this study may be related to Δ¹⁵N during digestion and absorption or an effect of the endogenous contribution to the protein synthesized in the samples.

Variation in the Δ¹⁵N of blood and plasma was observed over the 5-wk study. Because Δ¹⁵N has already been corrected for differences in dietary δ¹⁵N, this difference is most likely derived from the variation in protein turnover rates (Ben-David et al., 2012). Results from this study are consistent with a higher turnover rate of protein in plasma than in blood, which is in agreement with previous reports concerning a range of mammals (Hilderbrand et al., 1996; Lecomte et al., 2011; Ben-David et al., 2012).

The causes of the differences in turnover rates of Δ¹⁵N in blood and plasma are mainly due to their con-

Table 4. Correlation coefficients (r_p ; upper values) and P -values (lower values) between group means for feed conversion efficiency (FCE), plasma $\Delta^{15}\text{N}$, blood $\Delta^{15}\text{N}$, and wool $\Delta^{15}\text{N}$ (mean; $n = 4$)

Item	FCE	Plasma $\Delta^{15}\text{N}$	Blood $\Delta^{15}\text{N}$
Plasma $\Delta^{15}\text{N}$	-0.995 0.005		
Blood $\Delta^{15}\text{N}$	-0.964 0.036	0.961 0.039	
Wool $\Delta^{15}\text{N}$	-0.997 0.003	0.998 0.002	0.950 0.050

stituent proteins. The major proteins in plasma and blood are albumin and hemoglobin, respectively, and the half-life of albumin is much shorter (approximately 14 d) than that of hemoglobin (approximately 120 d; Rendell et al., 1987). This would suggest that 14 d may be a conservative lower limit for plasma protein turnover in sheep. The predicted blood ^{15}N half-life is approximately 20 d, when assuming a blood cell-to-plasma ratio of 45:55 and adopting 40 and 4 d as the respective half-lives of each tissue type (Lecomte et al., 2011).

Conclusions

Overall, the results are consistent with the potential of using $\Delta^{15}\text{N}$ as a rapid, low-cost biomarker of FCE in sheep, despite there being no effects of genetic treatment on FCE and $\Delta^{15}\text{N}$. Future studies on longer-term and complicated genetic and environmental interactions (e.g., different genotypes fed on different types and quantities of feed) should be explored, using both mature and growing animals, to confirm the value of using $\Delta^{15}\text{N}$ as a performance measure across entire sheep production systems.

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