

Plasma nitrogen isotopic fractionation and feed efficiency in growing beef heifers

N. M. Wheadon^{1,2}, M. McGee¹, G. R. Edwards² and R. J. Dewhurst^{1*}

¹Animal and Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, County Meath, Republic of Ireland

²Faculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln, New Zealand

(Submitted 10 July 2013 – Final revision received 19 November 2013 – Accepted 20 November 2013 – First published online 6 January 2014)

Abstract

Fractionation of N isotopes occurs in many metabolic reactions which causes tissue proteins to become enriched in ¹⁵N while urea (urine) is depleted in ¹⁵N relative to the diet. We investigated ¹⁵N enrichment of whole plasma and its relationship with feed conversion efficiency (FCE) in growing beef heifers (*n* 84) offered 2 kg/d of concentrates with grass silage *ad libitum*. Heifers were on average 299 (SD 48.3) d old and weighed 311 (SD 48.8) kg. Plasma was obtained on day 79 (*n* 84) of the experiment and from a subset of animals (*n* 20) on four occasions between days 16 and 79. Silage DM intake (DMI) averaged 4.1 (SD 0.74) kg/d and concentrate DMI was 1.72 kg/d. Mean mid-test live weight was 333 (SD 47.6) kg, daily gain was 0.53 (SD 0.183) kg, FCE (g live-weight gain/g DMI) was 0.09 (SD 0.028) and residual feed intake (RFI) was 0 (SD 0.428). N isotopic fractionation ($\Delta^{15}\text{N}$; plasma $\delta^{15}\text{N}$ – diet $\delta^{15}\text{N}$) averaged 3.58‰ on day 79 (*n* 84) and 3.90‰ for the subset of heifers. There was no relationship between $\Delta^{15}\text{N}$ and RFI. Plasma $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ were related to both FCE (negative) and animal weight (positive) for the whole population, and repeatable for the subset of animals over four time points. These relationships of $\Delta^{15}\text{N}$ with FCE and animal weight are consistent with the anticipated negative relationship with N-use efficiency. There is potential to use $\Delta^{15}\text{N}$ to provide rapid, low-cost estimates of FCE in cattle.

Key words: Beef cattle: Nitrogen-15: Isotopic fractionation: Feed efficiency

Livestock industries around the world face the partially contradictory objectives of increasing production of meat and milk to match growing demand, while also reducing environmental impact and greenhouse gas emissions⁽¹⁾, all within a resource-constrained setting. In particular, there is pressure to reduce N excretions because of new environmental legislation⁽²⁾. Improvements in feed efficiency are essential for the beef farmer because of increasing costs of production; feed is the largest variable cost and an important determinant of profitability in beef production⁽³⁾. Identification of, and genetic selection for, feed-efficient cattle is an important approach to achieve these multiple objectives⁽⁴⁾. Improvements in feed efficiency have already been successful in other species; for example, there was a 20% increase in feed efficiency in poultry between 1996 and 2003⁽⁵⁾.

Feed conversion ratio, defined as the ratio of feed intake: live-weight gain⁽⁶⁾, and feed conversion efficiency (FCE), defined as the units of output per unit of feed⁽⁷⁾, are traditional measures of feed efficiency commonly used in assessing beef cattle. Residual feed intake (RFI), defined as the difference between an animal's measured feed intake and expected intake based on its weight and average daily gain, is an

alternative method of measuring feed efficiency in beef cattle, and is independent of growth rate and live weight⁽⁸⁾.

Incorporation of feed efficiency measurements into cattle breeding strategies has been slow⁽⁴⁾. Feed efficiency is costly and laborious to measure, even with automated systems. It is necessary to record feed intake and growth over at least 70 d in order to have accurate estimates of efficiency⁽⁹⁾, and it is not possible to easily apply this approach to grazing animals⁽¹⁰⁾. There is also uncertainty about the composition of body reserves and live-weight change, as well as nutrient requirements for pregnancy which make these measurements even more difficult, especially with pregnant and (or) lactating animals⁽¹¹⁾.

More recently, there has been an increased interest in longer-term strategies of genetic and genomic selection for feed efficiency or its components such as digestive efficiency, metabolic efficiency or animal activity⁽¹²⁾. Success in identifying genetic and genomic associations with complex traits such as feed efficiency depends on being able to measure the phenotype in large numbers of animals representing a broad cross-section of genetic variation⁽¹³⁾. A number of studies have sought biochemical markers for feed efficiency (measured as RFI^(14–16)) in response to these difficulties. Unfortunately,

Abbreviations: $\Delta^{15}\text{N}$, plasma $\delta^{15}\text{N}$ – diet $\delta^{15}\text{N}$; DMI, DM intake; FCE, feed conversion efficiency; NUE, nitrogen-use efficiency; RFI, residual feed intake.

* **Corresponding author:** R. J. Dewhurst, email richard.dewhurst@sruc.ac.uk

these markers have only been poorly correlated with feed efficiency measurements. These relationships may have been influenced by physiological factors including dietary intake, digestibility, metabolism of nutrients, physical activity and thermoregulation⁽¹⁷⁾, and external factors such as environment and health⁽¹⁸⁾. Identification of phenotypic markers can also be used to aid in constructing diets to match the energy requirements of cattle and compare the efficiency of nutrient use for different diets.

We have investigated an alternative marker for feed efficiency based on the phenomenon of N isotopic fractionation⁽¹⁹⁾ that occurs during a number of metabolic pathways. N isotopic fractionation occurs when the incorporation of the natural isotopes ¹⁴N and ¹⁵N into products is slightly different from their ratio in precursors because of differences in the mass of small compounds containing either ¹⁴N or ¹⁵N. As a result of N isotopic fractionation, urine is depleted in ¹⁵N relative to the diet, while animal proteins are enriched^(20–22). In particular, enrichment of tissue protein occurs during transamination and deamination reactions in the conversion of excess dietary protein into urea⁽²³⁾, protein turnover and recycling^(22,24) and rumen metabolism⁽²⁵⁾. The magnitude of fractionation depends on the efficiency of assimilation⁽²⁴⁾ and metabolic rate⁽²⁶⁾ and varies between tissue type and metabolic pools^(27,28). N fractionation has been a useful predictor of N partitioning in non-ruminants, e.g. measurements in plasma protein from rats⁽²⁹⁾ and hair protein from horses, cattle and goats⁽³⁰⁾. There have also been previous reports of the use of N isotopes to investigate the effects of changing dietary protein in beef cattle⁽³⁰⁾, the mechanisms of fractionation in ruminal microbes⁽²⁵⁾ and for predicting nitrogen-use efficiency (NUE) in dairy cattle⁽³¹⁾.

N-use efficiency, defined as the efficiency of converting feed N into animal protein N, is an important component of overall feed efficiency⁽³²⁾, as muscle protein is the most valuable component of the carcass in beef cattle. Since body composition was not measured in the present study, we have related N isotopic fractionation to overall FCE. We expect a strong relationship between NUE and FCE, particularly when offering the same diet. The adjustments involved in calculating RFI and other more complex measures of feed efficiency make them less likely to be related to N isotopic fractionation than simple measures of input/output such as FCE investigated in this work.

The objective of the present study was to investigate the relationship between N isotopic fractionation, measured in plasma, and estimates of FCE in growing beef heifers. We also investigated the repeatability of N isotopic fractionation over a 3-month period and hypothesised that there would be no change in the relationship between N isotopic fractionation and FCE over time.

Materials and methods

Animals and their management

All animal procedures performed in the present study were conducted under experimental licence from the Irish

Department of Health and Children in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendment of Cruelty to Animals Act 1876) Regulation 2002 and 2005. The present study used plasma samples from eighty-four beef heifers (sixty-two Simmental and twenty-two Simmental × Holstein-Friesian) sampled on one occasion towards the end of a period of recording FCE. A subset of twenty heifers from the same population (fifteen Simmental and five Simmental × Holstein-Friesian) was sampled over four time points spaced across the FCE recording period. Further details of the original study have been described by Lawrence *et al.*⁽¹⁶⁾. The heifers were on average 299 (SD 48.3) d old and weighed 311 (SD 48.8) kg at the start of the experiment. They were housed in pens of four to six animals (lying area 2.87 m²/animal) in a shed with slatted floors and adapted to their diet for 3 weeks before a 12-week recording period. Heifers were individually offered grass silage *ad libitum* and were fed 2 kg/d concentrates (at 08.00 hours) through Calan gates (American Calan, Inc.). Daily feed intakes were recorded and animals were weighed at 3-week intervals.

Sample collection and analysis

Blood samples were obtained by jugular venepuncture from all animals on day 79, and from the subset of twenty animals on days 16, 37, 58 and 79 of the experimental period. The former samples were collected into 4 ml evacuated tubes containing sodium citrate (Greiner Vacuette; Cruinn Diagnostics), while samples from the subset of twenty animals were collected separately into 9 ml evacuated tubes containing lithium heparin (Greiner Vacuette; Cruinn Diagnostics). Blood samples were then centrifuged (2500 g, 20 min, 4°C), and whole plasma was stored at –20°C until analysis.

Grass silage and concentrate offered was sampled three and two times weekly, and composited weekly and fortnightly, respectively. These were analysed according to the methods described by Owens *et al.*⁽³³⁾ with the exception that the DM content of the grass silage was determined after drying at 40°C for 48 h. Samples of the concentrates and grass silage (*n* 20; all in duplicate), as well as plasma samples, measured in whole plasma (*n* 164 plus 10% duplicates) were analysed for ¹⁵N content by isotope-ratio MS (Iso-Analytical Limited). N-15 results are expressed in δ units relative to standard air ($\delta^{15}\text{N}$, ‰). N isotopic fractionation, the difference between $\delta^{15}\text{N}$ for plasma and $\delta^{15}\text{N}$ for the diet, is termed $\Delta\delta^{15}\text{N}$, or simply $\Delta^{15}\text{N}$. Average diet $\delta^{15}\text{N}$ was calculated for each animal by weighting $\delta^{15}\text{N}$ for silage and $\delta^{15}\text{N}$ for concentrate based on N intake (g/d) of each diet component.

Statistical analysis

RFI was calculated as the difference between actual DM intake (DMI) and expected DMI using regression models to predict DMI⁽¹⁶⁾. The relationships between plasma $\delta^{15}\text{N}$, $\Delta^{15}\text{N}$, FCE, RFI and $W^{0.75}$ were described using linear regression (GenStat release 10.1; Lawes Agricultural Trust (Rothamsted Experimental Station), 2007) using breed as a factor in the model. ANOVA was used to establish the effect of breed and sire on N isotopic



Table 1. Average chemical composition of feed samples collected over the experimental period

	Diet component	
	Grass silage	Concentrate
DM (g/kg)	244.3	85.79
pH	3.9	ND
Composition of DM (g/kg DM, unless otherwise stated)		
<i>In vitro</i> DMD*	744	858
<i>In vitro</i> DOMD†	688	ND
OMD‡	762	ND
Ash	98	85
Crude protein	136	140
NDF	511	215
Starch	ND	269
Fermentation characteristics (g/kg DM)		
Lactic acid	43	ND
Acetic acid	80	ND
Propionic acid	4.9	ND
Butyric acid	12.9	ND
Ethanol	57.4	ND
$\text{NH}_3\text{-N}$ (g/kg total N)	73	ND
$\delta^{15}\text{N}$ (‰)	5.74	3.20

ND, not determined; DMD, DM digestibility; DOMD, digestible organic matter in total DM; OMD, organic matter digestibility; NDF, neutral-detergent fibre.

* Measured *in vitro*.

† Measured *in vitro*.

‡ Measured *in vitro*.

fractionation and feed efficiency. Pearson's correlations were used to show the relationship of N isotopic fractionation between time points, and a REML repeated-measures model was used to analyse changes in the relationship between FCE and N isotopic fractionation over time.

Results

A total of eighty-six heifers commenced the experiment, but values were excluded from one sick animal and one extreme outlier (confirmed using Cook's test). These exclusions had no effect on the relationships identified. The average chemical composition of the grass silage and concentrates across the experiment is described in Table 1. Across the whole population, average DMI was 5.82 (SD 0.74) kg/d, silage DMI averaged 4.1 (SD 0.74) kg/d and concentrate intake was 1.72 kg/d, so that the forage proportion of total DMI averaged 0.70 (SD 0.038) g/g. Mean mid-test live weight was 333 (SD 47.6) kg, average daily gain was 0.53 (SD 0.183) kg, FCE (g live-weight gain/g DMI) was 0.09 (SD 0.028) and average RFI was 0 (SD 0.428). For the subset of twenty heifers, the

average DMI was 5.82 (SD 0.59) kg/d, silage DMI averaged 4.1 (SD 0.59) kg/d and concentrate was 1.72 kg/d, so forage proportion of the total DMI averaged 0.70 (SD 0.031) g/g. Mean mid-test live weight was 339 (SD 53.7) kg, average daily gain was 0.55 (SD 0.164) kg, average FCE was 0.10 (SD 0.030) and RFI was -0.079 (SD 0.472).

Although feed samples were drawn from the same batches, there was a slight variation in $\delta^{15}\text{N}$ values, so the average values were used in calculating $\Delta^{15}\text{N}$. The average $\delta^{15}\text{N}$ values of the concentrates and grass silage were 3.20 (SD 0.189) and 5.74 (SD 0.103)‰, and the weighted value for the whole diet was 4.96 (SD 0.082)‰ for the subset of twenty animals and 4.95 (SD 0.098)‰ for all the eighty-four animals. Plasma $\delta^{15}\text{N}$ for each of the four time points averaged 8.85 (SD 0.43), 8.80 (SD 0.40), 8.84 (SD 0.41) and 8.69 (SD 0.48), respectively, ranging from a 3.04 to 5.11 unit enrichment relative to the diet. The average $\delta^{15}\text{N}$ of plasma for all the eighty-four animals at day 79 was 8.53 (SD 0.364)‰, ranging from a 3.51 to 5.14 unit enrichment relative to the diet.

Although there was a small range in overall diet $\delta^{15}\text{N}$ (a consequence of the differences in the forage:concentrate ratio consumed), there was no significant relationship between diet $\delta^{15}\text{N}$ and plasma $\delta^{15}\text{N}$ for the whole population or any of the repeated time points for the subset of heifers. Linear regression analysis using data from all animals at day 79, as well as for the subset of twenty animals at each of the four time points, showed a significant negative relationship between plasma $\delta^{15}\text{N}$ and FCE in each case (Table 2). N isotopic fractionation ($\Delta^{15}\text{N}$) was also negatively related to FCE for all the sample time points (Fig. 1(a)–(e), equations 1–5). The subset showed stronger relationships than those observed for the whole population:

$$\text{Day 79 } (n = 84): \Delta^{15}\text{N} = 4.216 \text{ (SE 0.101)} \\ - 7.36 \text{ (SE 1.10)} \times \text{FCE (g/g)}. \quad (1)$$

$$\text{Day 16 } (n = 20): \Delta^{15}\text{N} = 4.781 \text{ (SE 0.231)} \\ - 9.33 \text{ (SE 2.32)} \times \text{FCE (g/g)}. \quad (2)$$

$$\text{Day 37 } (n = 20): \Delta^{15}\text{N} = 4.743 \text{ (SE 0.197)} \\ - 9.49 \text{ (SE 1.98)} \times \text{FCE (g/g)}. \quad (3)$$

Table 2. Coefficients of determination (r^2) or coefficients of multiple determination (R^2) for the relationships between feed conversion efficiency (g live-weight gain/kg DM intake) and plasma $\delta^{15}\text{N}$, $\Delta^{15}\text{N}$ (plasma $\delta^{15}\text{N}$ - diet $\delta^{15}\text{N}$) and mid-test $W^{0.75}$, with average standard errors*

	Day of experiment					SEM
	16	37	58	79 (n 20)	79 (n 84)	
Plasma $\delta^{15}\text{N}$	0.52	0.63	0.69	0.59	0.28	0.284
$\Delta^{15}\text{N}$	0.47	0.56	0.64	0.56	0.35	0.284
Plasma $\delta^{15}\text{N}$ + mid-test $W^{0.75}$	0.81	0.83	0.84	0.74	0.44	0.22
$\Delta^{15}\text{N}$ + mid-test $W^{0.75}$	0.72	0.71	0.75	0.68	0.41	0.244

* All relationships were significant at the $P < 0.001$ level.

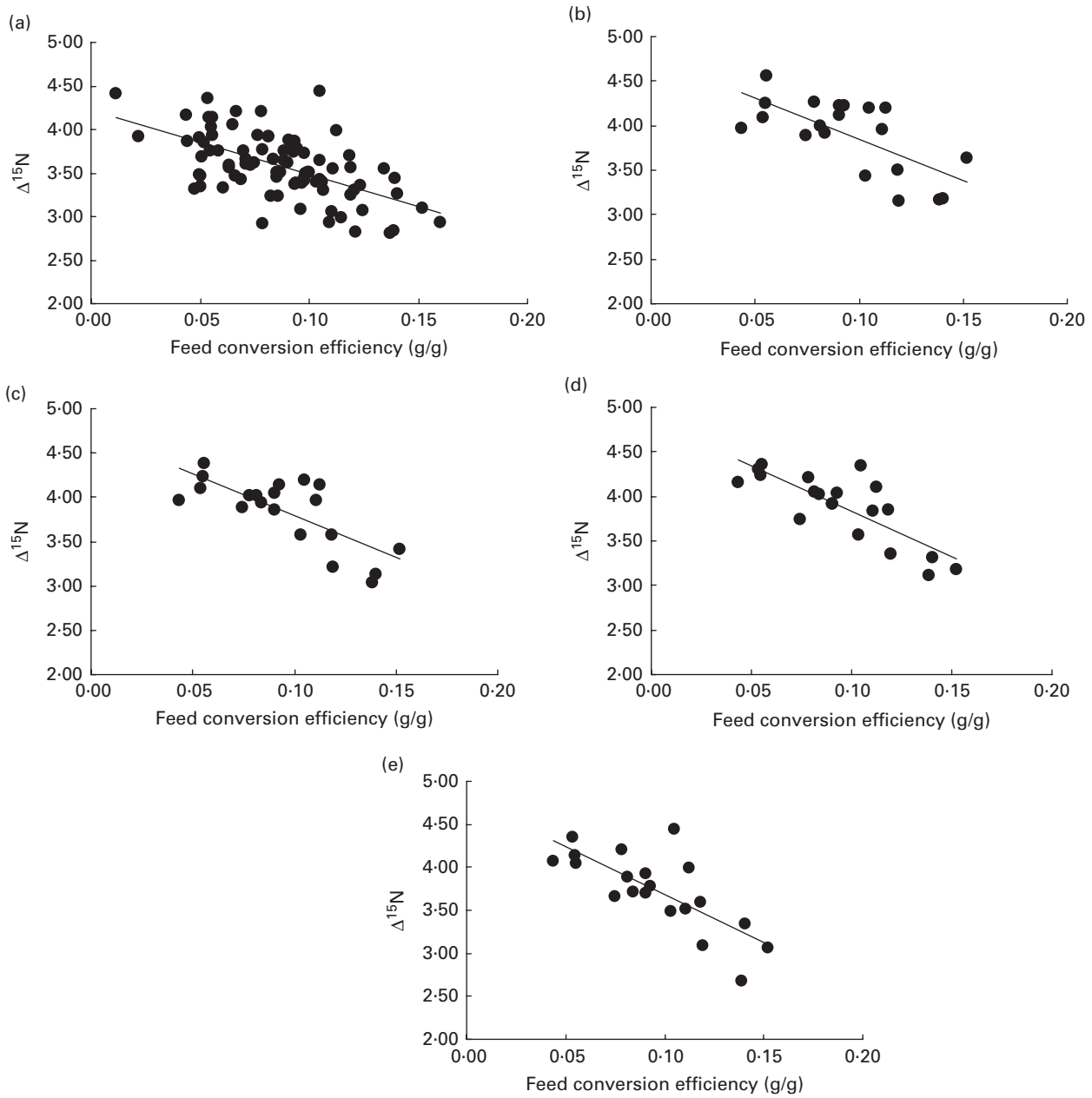


Fig. 1. Relationship between nitrogen isotopic fractionation ($\Delta^{15}\text{N}$; the difference between plasma $\delta^{15}\text{N}$ and diet $\delta^{15}\text{N}$) and feed conversion efficiency (g live-weight gain/g DM intake) for (a) all animals at day 79 (n 84; R^2 0.35) and (b)–(e) a subset of twenty animals at days 16 (n 20; R^2 0.47), 37 (n 20; R^2 0.56), 58 (n 20; R^2 0.64) and 79 (n 20; R^2 0.56).

$$\begin{aligned} \text{Day 58 } (n = 20): \Delta^{15}\text{N} &= 4.854 \text{ (SE } 0.179) \\ &- 10.25 \text{ (SE } 1.80) \times \text{FCE (g/g)}. \end{aligned} \tag{4}$$

$$\begin{aligned} \text{Day 79 } (n = 20): \Delta^{15}\text{N} &= 4.789 \text{ (SE } 0.232) \\ &- 11.11 \text{ (SE } 2.33) \times \text{FCE (g/g)}. \end{aligned} \tag{5}$$

There was no significant difference between the slopes on different days ($P=0.848$). Plasma $\delta^{15}\text{N}$ measurements from the same animals in the subset were significantly correlated

over adjacent time points ($P < 0.001$ correlation between all days; average r 0.96), and the degree of correlation for individuals decreased with greater time separation between samplings (e.g. r 0.84 between the twenty animals on the first and last sampling dates).

There was no significant relationship between FCE and DMI (r^2 0.06; $P=0.16$). There was a weak (r^2 0.10), but significant ($P=0.002$), negative relationship between FCE and mid-test metabolic weight ($W^{0.75}$). Nonetheless, adding $W^{0.75}$ in a multiple regression analysis explained an additional and highly significant part of the variation in plasma $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ across all time points (Table 2). There was no significant

relationship either between plasma $\delta^{15}\text{N}$ and RFI ($P=0.21$; r^2 0.07), or between $\Delta^{15}\text{N}$ and RFI ($P=0.82$; r^2 0).

There was a significant difference in FCE between breeds in the present study ($P<0.001$). Pure Simmental heifers had lower FCE (0.076 (SD 0.003)) than Holstein-Friesian \times Simmental heifers (0.119 (SD 0.004)). Simmental heifers also had significantly higher plasma $\delta^{15}\text{N}$ ($P<0.001$) and $\Delta^{15}\text{N}$ ($P<0.001$) than Holstein Friesian \times Simmental cross heifers (8.63 (SD 0.04) and 8.22 (SD 0.06); 4.38 (SD 0.04) and 3.92 (SD 0.06), respectively). Despite significant differences in FCE and N isotopes between breeds, there was no significant effect on the relationship between FCE and $\delta^{15}\text{N}$ or $\Delta^{15}\text{N}$. FCE (g/g) also differed across sire groups ($P<0.001$), ranging from 0.06 (SD 0.010) to 0.13 (SD 0.001). There were fifteen sires for the larger group and three sires for the subset group. Plasma $\delta^{15}\text{N}$ and, as a result, plasma $\delta^{15}\text{N}$ – diet $\delta^{15}\text{N}$ were significantly different between the sire groups ($P<0.001$ and $P<0.001$). Plasma $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ ranged from 8.17 (SD 0.12) to 8.97 (SD 0.11) and 3.81 (SD 0.11) to 4.69 (SD 0.10) for different sire groups, respectively. Plasma $\delta^{15}\text{N}$ and $\Delta^{15}\text{N}$ tended to be higher in sire groups with lower FCE. FCE and average daily gain were slightly higher for the subset of twenty heifers compared with the whole population ($P<0.001$ and $P<0.001$, respectively).

Discussion

NUE in cattle can be improved by increasing N retention in muscle at a similar intake, by reducing N intake at a similar N retention, or by a combination of both. Live-weight gain is the main driver for N retention in growing animals, and as muscle protein is the most valuable component of the carcass in beef cattle, it is essential to identify animals with higher NUE which will aid in lowering the impact of detrimental N emissions to the environment⁽¹⁾. Phenotypic markers such as N isotopic fractionation used in the present study have

potential to be used to measure feed efficiency in cattle where diet composition or intake cannot be recorded. This will accelerate collection of feed efficiency data for large numbers of animals in breeding programmes.

Comparison of nitrogen isotopic fractionation with earlier studies

N isotopic fractionation ($\Delta^{15}\text{N}$) between whole plasma and the diet overall averaged 3.74 in the present study (range 3.04–5.11‰), which is lower than the range measured previously with growing or mature ruminants (3.80–6.94‰ for treatment means; Table 3). The plasma urea fraction is generally depleted in $\delta^{15}\text{N}$ compared with both the diet and the plasma protein fraction⁽²²⁾. N fractionation can be measured in the plasma protein or urea N fraction of plasma; however, in the present study, whole plasma was used because the urea fraction contains less than 1% of the N in plasma, so the effect on results in the present study would be negligible. For the same reason, we calculate that the fact that the highest $\Delta^{15}\text{N}$ in the literature (Table 3) was for plasma protein is purely coincidental. There was only a weak relationship (r^2 0.1) between plasma urea N and FCE in the present study, which taken together with the low level of urea N relative to protein N in plasma suggests that the urea fraction of plasma would not affect the relationship between FCE and plasma $\delta^{15}\text{N}$.

While most previous studies provided only limited descriptions of the animals, many involved mature animals that would have ceased growing and so use dietary N relatively inefficiently, resulting in a high level of isotopic fractionation ($\Delta^{15}\text{N}$). Lower $\Delta^{15}\text{N}$ were observed in studies with suckling young and in most, but not all, studies with lactating ruminants. It would be expected that these more productive animals would be using N more efficiently, partitioning a higher proportion of N to muscle or milk protein and less to urea.

Table 3. Literature values for nitrogen isotopic fractionation in ruminants ($\Delta^{15}\text{N}$; $\delta^{15}\text{N}$ in blood minus $\delta^{15}\text{N}$ in the diet)

Reference	Physiological state	Animal description	Diet	$\Delta^{15}\text{N}$	Sample type
Present study*	Growing	Growing heifers	Grass silage/concentrates	3.58	Plasma
Present study†	Growing	Growing heifers	Grass silage/concentrates	3.90	Plasma
Steele & Daniel ⁽³⁶⁾	Growing/mature	Angus steers	Ryegrass/white clover silage	4.2	Blood
Sponheimer <i>et al.</i> ^{(30)‡}	Growing/mature	Cattle	Lucerne hay	4	Blood
Koyama <i>et al.</i> ⁽³⁷⁾	Growing/mature	Beef cattle	Rice straw	3.8	Blood
Sponheimer <i>et al.</i> ^{(30)‡}	Growing/mature	Goats	Lucerne hay	4.7	Plasma
Sutoh ⁽³⁸⁾	Mature	Goats, 2-year-old females	Lucerne hay cubes	4.8	Plasma
Sutoh <i>et al.</i> ⁽³⁹⁾	Mature	Wethers, 4 years old	Lucerne hay cubes	6.94	Plasma protein
Darr & Hewitt ⁽⁴⁰⁾	Mature	Deer, 2-year-old males	Lucerne	5.67	Serum
Darr & Hewitt ⁽⁴⁰⁾	Mature	Deer, 2-year-old males	Lucerne/maize (3:2)	6.2	Serum
Koyama <i>et al.</i> ⁽³⁷⁾	Lactating	Beef cows, milking	Pasture	2.59	Blood
Sutoh <i>et al.</i> ⁽³⁹⁾	Lactating	Lactating dairy cows	Forage/concentrates (1:1)	2.37	Plasma
Cheng <i>et al.</i> ⁽⁴¹⁾	Lactating	Lactating dairy cows	Grass	3.19	Plasma
Jenkins <i>et al.</i> ^{(42)‡}	Lactating	Sheep, early lactation	Hay	5.1	Plasma
Jenkins <i>et al.</i> ^{(42)‡}	Lactating	Sheep, early lactation		4.5	Plasma
Jenkins <i>et al.</i> ⁽⁴²⁾	Neonate	Deer calves, 12–14 d old	Milk	1.9	Plasma
Jenkins <i>et al.</i> ⁽⁴²⁾	Neonate	Lambs, 12–14 d old	Milk	3	Plasma
Jenkins <i>et al.</i> ⁽⁴²⁾	Neonate	Moose calves, <3 months old	Milk	1.5	Plasma

* Whole population (n 84).

† Subset heifers averaged over time (n 20).

‡ Unpublished results cited by Robbins *et al.*⁽⁴³⁾.

Repeatability of nitrogen isotopic fractionation measurements

In the present study, we demonstrated a consistent and repeatable strong relationship between N isotopic fractionation and FCE that will be useful for application of the approach in animal breeding. The correlation between plasma $\delta^{15}\text{N}$ at the different time points was also usefully strong. It appears that FCE remained constant over the measurement period and $\Delta^{15}\text{N}$ values remained stable despite the natural variation in growth rate that may have been increased because of heifers entering puberty at different stages during the experiment⁽³⁴⁾.

Relationship of nitrogen isotopic fractionation with feed conversion efficiency

N isotopic fractionation ($\Delta^{15}\text{N}$) was a good indicator of FCE in the present study, and it seems likely that this relationship was driven by the partitioning of N between live-weight gain and excretion in the urine. The negative relationship between $\Delta^{15}\text{N}$ and FCE is consistent with the increased $\Delta^{15}\text{N}$ when cattle and goats were fed diets containing higher protein levels⁽³⁰⁾. The relationship between $\Delta^{15}\text{N}$ and FCE was probably stronger for the subset of twenty animals because they were more homogeneous (lower SD for live weight) and genetically more similar. Plasma $\delta^{15}\text{N}$ differed between the breed and sire groups, providing preliminary evidence that it will be possible to use this approach to select for beef cattle with higher FCE. N isotopic fractionation only explained 1% of the variance of RFI. As expected, N isotopic fractionation was more related to simple measures of input and output (i.e. FCE) than complex measures of efficiency such as RFI.

Since the composition of live-weight gain was not measured in the present study, it is likely that some of the unexplained variation in the relationships between plasma $\delta^{15}\text{N}$ or $\Delta^{15}\text{N}$ and FCE results from the variation in the relationship between NUE and FCE. The inclusion of $W^{0.75}$ accounted for some of the variation in the relationship between $\Delta^{15}\text{N}$ and FCE (Table 2) because the N content of live-weight gain declines as animals grow⁽³⁵⁾. Average values for the protein content of live-weight gain are 6.3% lower for 450 kg cattle in comparison with 250 kg cattle⁽³⁵⁾, implying a reduction in NUE at the same FCE. Fractionation of N isotopes in the rumen could explain additional variation^(25,36), though the strong relationships with FCE and use of a consistent diet suggest that any effect would have been small.

N isotopic fractionation ($\Delta^{15}\text{N}$) explained more variation in FCE than single blood metabolites or hormones that were used to predict RFI in earlier studies. In these animals, Lawrence *et al.*⁽¹⁶⁾ found no significant relationships between blood metabolites and RFI, except for creatinine. Kelly *et al.*⁽¹⁵⁾ reported weak relationships between feed conversion ratio and plasma leptin (r^2 0.23), urea (r^2 0.18) and NEFA (r^2 0.10) in heifers, while Richardson *et al.*⁽¹⁴⁾ found weak relationships between feed conversion ratio and plasma glucose (r^2 0.21), aspartate aminotransferase (r^2 0.20) and albumin (r^2 0.23) in steers.

The partitioning of N between protein (muscle tissue or milk) and urea is affected by protein supply and protein

quality^(22,29), and both aspects have been used to explain differences in N isotopic fractionation. The first effect suggests that as dietary protein supply increases, an increasing proportion of N is directed to urine, so that fractionation increases. The second effect emphasises increased fractionation as a result of decreased protein quality and consequent increased losses as urinary N. In the present study, animals received the same diet, so the effects must be mainly due to the between-animal variation in feed utilisation. The between-animal variation in N partitioning depends on the ability of individual animals to utilise protein, which will depend on their genetic make-up and management history. There was a weak relationship between plasma urea N and FCE in the present study and a low level of urea N relative to protein N in plasma, which suggests that the urea fraction of plasma would not affect the relationship between FCE and plasma $\delta^{15}\text{N}$. N fractionation in the present study was able to predict the animal variation associated with FCE.

Conclusion

We have demonstrated a highly significant negative relationship between N isotopic fractionation between plasma and the diet ($\Delta^{15}\text{N}$) and feed efficiency in growing beef heifers. This N isotopic fractionation effect changed only slowly over time, which would be useful for application in cattle breeding. N isotopic fractionation may be useful in evaluating feed efficiency without measuring feed intake, and even diet composition, provided that animals are fed with identical diets, and be used to compare the nutrient-use efficiency of different feeds.

Acknowledgements

The authors acknowledge the skilled technical assistance of J. Larkin and E. Mulligan.

The present study was financially supported by the European Union (Marie Curie International Reintegration Grant FP7-PEOPLE-IRG-2008-239241) and as part of the Department of Agriculture, Food and the Marine grant in aid to Teagasc. The funders had no role in the design, analysis or writing of this article.

The authors' responsibilities were as follows: N. M. W., M. M., G. R. E. and R. J. D. were involved in the design of the study, data analysis and preparation of the manuscript; N. M. W. conducted additional laboratory analysis and prepared the first draft of the manuscript. All authors read and approved the final manuscript.

None of the authors has any conflict of interest.

References

- Gill M, Smith P & Wilkinson JM (2010) Mitigating climate change: the role of domestic livestock. *Animal* **4**, 323–333.
- Powell JM, MacLeod M, Vellinga TV, *et al.* (2013) Feed-milk–manure nitrogen relationships in global dairy production systems. *Livest Sci* **152**, 261–272.
- Ashfield A, Crosson P & Wallace M (2013) Simulation modelling of temperate grassland based dairy calf to beef production systems. *Agr Syst* **115**, 41–50.



4. Herd RM, Oddy VH & Richardson EC (2004) Biological basis for residual feed intake in beef cattle. 1. Review of potential mechanisms. *Aust J Exp Agr* **44**, 423–430.
5. Case LA, Wood BJ & Miller SP (2012) The genetic parameters of feed efficiency and its component traits in the turkey (*Meleagris gallopavo*). *Genet Sel Evol* **44**, 2.
6. Archer JA, Richardson EC, Herd RM, *et al.* (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. *Aust J Agr Res* **50**, 147–161.
7. Crews DH (2005) Genetics of efficient feed utilization and national cattle evaluation: a review. *Genet Mol Res* **4**, 152–165.
8. Koch RM, Swiger LA, Chambers D, *et al.* (1963) Efficiency of feed use in beef cattle. *J Anim Sci* **22**, 486–494.
9. Herd RM, Archer JA & Arthur PF (2003) Reducing the cost of beef production through genetic improvement in residual feed intake: opportunity and challenges to application. *J Anim Sci* **81**, E9–E17.
10. Archer JA, Arthur PF, Herd RM, *et al.* (1997) Optimum post weaning test for measurement of growth rate, feed intake and feed efficiency in British breed cattle. *J Anim Sci* **75**, 2024–2032.
11. Dewhurst RJ, Moorby JM, Dhanoa MS, *et al.* (2000) Effects of altering energy and protein supply to dairy cows during the dry period. 1. Intake, body condition, and milk production. *J Dairy Sci* **83**, 1782–1794.
12. Moore SS, Mujibi FD & Sherman EL (2009) Molecular basis for residual feed intake in beef cattle. *J Anim Sci* **87**, E41–E47.
13. Robinson DL & Oddy VH (2004) Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. *Livest Prod Sci* **90**, 255–270.
14. Richardson EC, Herd RM, Archer JA, *et al.* (2004) Metabolic differences in Angus steers divergently selected for residual feed intake. *Aust J Exp Agr* **44**, 441–452.
15. Kelly AK, McGee M, Crews DH, *et al.* (2010) Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. *J Anim Sci* **88**, 109–123.
16. Lawrence P, Kenny DA, Earley B, *et al.* (2012) Grazed grass herbage intake and performance of beef heifers with predetermined phenotypic residual feed intake classification. *Animal* **6**, 1648–1661.
17. Herd RM & Arthur PF (2009) Physiological basis for residual feed intake. *J Anim Sci* **87**, E64–E71.
18. Arthur PF, Archer JA & Herd RM (2004) Feed intake and efficiency in beef cattle: overview of recent Australian research and challenges for the future. *Aust J Exp Agr* **44**, 361–369.
19. Macko SA, Fogel-Estep ML, Engel MH, *et al.* (1986) Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. *Geochim Cosmochim Acta* **50**, 2143–2146.
20. Minagawa M & Wada E (1984) Stepwise enrichment of ¹⁵N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta* **48**, 1135–1141.
21. Sutoh M, Koyama T & Yoneyama T (1987) Variations of natural ¹⁵N abundance in the tissues and digesta of domestic animals. *Radioisotopes* **36**, 74–77.
22. Poupin N, Bos C, Mariotti F, *et al.* (2011) The nature of the dietary protein impacts the tissue-to-diet ¹⁵N discrimination factors in laboratory rats. *PLoS One* **11**, e28046.
23. Balter V, Simon L & Fouillet H (2006) Box-modelling of ¹⁵N/¹⁴N in mammals. *Ecophysiology* **147**, 212–222.
24. Martinez del Rio C & Carleton SA (2012) How fast and how faithful: the dynamics of isotopic incorporation into animal tissues. *J Mammal* **93**, 353–359.
25. Wattiaux MA & Reed JD (1995) Fractionation of nitrogen isotopes by mixed ruminal bacteria. *J Anim Sci* **73**, 257–266.
26. Smith JJ, Millar JS, Longstaffe FJ, *et al.* (2010) The effect of metabolic rate on stable carbon and nitrogen isotope compositions in deer mice, *Peromyscus maniculatus*. *Can J Zool* **88**, 36–42.
27. De Niro MJ & Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* **45**, 341–351.
28. Caut S, Angulo E & Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol* **46**, 443–453.
29. Sick H, Roos N, Saggau E, *et al.* (1997) Amino acid utilization and isotope discrimination of amino acid nitrogen in nitrogen metabolism of rat liver *in vivo*. *Z Ernahrungswiss* **36**, 340–346.
30. Sponheimer M, Robinson T, Ayliffe L, *et al.* (2003) Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}\text{N}$ values from a controlled feeding study. *Int J Osteoarchaeol* **13**, 80–87.
31. Cheng L, Kim EJ, Merry RJ, *et al.* (2011) Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *J Dairy Sci* **94**, 2031–2041.
32. Wheadon NM, Edwards GR & Dewhurst RJ (2012) Comparison of nitrogen-use efficiency and energy conversion efficiency as measures of feed conversion efficiency in Holstein-Friesian cows over an entire lactation cycle. In *Proceedings of the British Society of Animal Science*. Edinburgh: British Society of Animal Science.
33. Owens D, McGee M, Boland T, *et al.* (2008) Intake, rumen fermentation and nutrient flow to the omasum in beef cattle fed grass silage fortified with sucrose and/or supplemented with concentrate. *Anim Feed Sci Technol* **144**, 23–43.
34. Ciccioli NH, Charles-Edwards SL, Floyd C, *et al.* (2005) Incidence of puberty in beef heifers fed high-or low-starch diets for different periods before breeding. *J Anim Sci* **83**, 2653–2662.
35. Agricultural Research Council (1980) *The Nutrient Requirements of Ruminant Livestock*. Farnham Royal: Commonwealth Agricultural Bureaux.
36. Steele KW & Daniel RM (1978) Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ¹⁵N for tracer studies. *J Agr Sci* **90**, 7–9.
37. Koyama T, Madoka S & Tadakatu Y (1985) Fractionation of nitrogen isotopes by domestic animals. *Jpn J Zootechnical Sci* **56**, 361–363.
38. Sutoh M (1989) Comparison of natural abundance of ¹⁵N between diet, rumen contents and blood plasma. *Asian Austral J Anim Sci* **2**, 548–549.
39. Sutoh M, Obara Y & Yoneyama T (1993) The effect of feeding regimen and dietary sucrose supplementation on natural abundance of ¹⁵N in some components of ruminal fluid and plasma of sheep. *J Anim Sci* **71**, 226–231.
40. Darr RL & Hewitt DG (2008) Stable isotope trophic shifts in white-tailed deer. *J Wildlife Manage* **72**, 1525–1531.
41. Cheng L, Sheahan AJ, Gibbs SJ, *et al.* (2013) Technical Note: nitrogen isotopic fractionation can be used to predict nitrogen-use efficiency in dairy cows fed temperature pasture. *J Anim Sci* **91**, 5785–5788.
42. Jenkins SG, Partridge ST, Stephenson TR, *et al.* (2001) Nitrogen and carbon isotope fractionation between mothers, neonate and nursing offspring. *Oecologia* **129**, 336–341.
43. Robbins T, Felicetti LA & Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* **144**, 534–540.