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## BRIEF COMMUNICATION: Investigation of N isotopic fractionation in dairy cows using milk samples collected at the morning and afternoon milkings

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### INTRODUCTION

Conversion of feed protein into milk protein is a basis of sustainable dairy production systems from both economic and environmental perspectives. This project addresses the need for rapid screening tools to support nutritional and breeding strategies to improve the efficiency of converting feed into milk protein. The approach is based on the phenomenon of N isotopic fractionation, whereby N sinks become relatively enriched or depleted in <sup>15</sup>N, as a result of differential incorporation during various biochemical reactions. Macko *et al.* (1986) showed that N isotopic fractionation was related to the deamination and transamination of amino acids. Sick *et al.* (1997) conducted a feeding experiment with rats and found that plasma protein was enriched and plasma urea was depleted in <sup>15</sup>N relative to the diet. Furthermore, Robbins *et al.* (2005) suggested that dietary protein quality had significant effects on nitrogen isotopic discrimination in mammals and birds. More recent studies showed that N isotopic fractionation occurs between urine and milk, and is indicative with N partitioning of dairy cows (Cheng, 2008; Cheng and Dewhurst, 2009). However, this approach still requires separate collection of milk and urine. Therefore, this study investigated the potential of using N isotopic fractionation of protein and non-protein fractions of milk samples only, which are easily collected, as an indicator of N partitioning.

### MATERIALS AND METHODS

Milk samples were collected from ten Holstein-Friesian cows (mean 140 days in milk), that were rotationally grazing summer ryegrass/white clover pasture and receiving no supplementary feed on Ballydague Farm in Ireland. Fresh pasture was allocated every 48 hours. Cows were milked twice daily and separate samples of morning and afternoon milk were collected, preserved with a lactab (Thompson and Capper Ltd., Runcorn, UK) and stored frozen at -20°C. Milk samples were then thawed, defatted by centrifugation at 11,500 rpm for 20 minutes at 4°C and protein precipitated using

acetone (4:1 acetone: milk (vol/vol)); left to stand for 2 hours, then centrifuged at 14,000 rpm for 10 minutes at 4°C; before washing with acetone. Samples of whole milk, the protein pellet and the supernatant, being the non-protein-N fraction, were freeze-dried and prepared for <sup>15</sup>N and N concentration analysis using a continuous flow isotope ratio mass spectrometer (PDZ Europa, Crewe, UK). Nitrogen-<sup>15</sup> content (<sup>15</sup>N) is conventionally presented in delta units (δ<sup>15</sup>N) by expressing the <sup>15</sup>N/<sup>14</sup>N ratio of the sample relative to the <sup>15</sup>N/<sup>14</sup>N ratio of air, on a per-thousand basis (‰). Comparisons of the <sup>15</sup>N content in the different milk fractions and between morning and evening samples were conducted using one-way analysis of variance and linear regression using GenStat, Version 10 (VSN International Ltd., Hemel Hempstead, Hertfordshire, UK).

### RESULTS AND DISCUSSION

The <sup>15</sup>N content (δ<sup>15</sup>N, ‰) of whole milk ranged from 6.30 to 7.24. The mean <sup>15</sup>N content in the protein fraction was 7.05 (Standard deviation (SD) = 0.25), which was significantly (P < 0.001) higher than in whole milk (6.81; SD = 0.265), whilst the <sup>15</sup>N content in the non-protein-N fraction was significantly (P < 0.001) lower (1.61; SD = 0.448). There were significant differences between morning and evening samples in content of <sup>15</sup>N in protein, non-protein-N and whole milk, values for morning milk being higher for milk protein and lower for milk non-protein-N (Table 1). There were significant relationships between individual morning and evening values for all three variables (Table 2).

The enrichment of <sup>15</sup>N in milk protein and depletion of <sup>15</sup>N in milk non-protein-N, relative to whole milk, is comparable with the earlier observations of enrichment of whole milk and depletion of urine relative to feed (Cheng & Dewhurst, 2009). The numerical difference in δ<sup>15</sup>N between protein pellet and milk non-protein-N (Mean = 5.4‰; Range: 4.6 to 6.4‰) was similar to the difference in δ<sup>15</sup>N between milk and urine (Mean = 6.2‰; Range: 4.3 to 7.6‰) for treatment

**TABLE 1:** Effect of morning or afternoon sampling on the relative  $^{15}\text{N}$  content ( $\delta^{15}\text{N}$ , ‰) of whole milk, milk protein and milk non-protein-N fractions isolated from milk samples obtained from ten grazing dairy cows. SED = Standard error of difference.

Milk fraction	Time of milking		SED	P value
	Morning	Afternoon		
Whole milk $\delta^{15}\text{N}$	6.90	6.72	0.04	0.002
Milk protein $\delta^{15}\text{N}$	7.13	6.98	0.05	0.009
Milk non-protein-N $\delta^{15}\text{N}$	1.36	1.87	0.05	<0.001

**TABLE 2:** Linear regression equations relating  $^{15}\text{N}$  content ( $\delta^{15}\text{N}$ , ‰) in morning samples (Y) to  $^{15}\text{N}$  content ( $\delta^{15}\text{N}$ , ‰) in afternoon samples (X). Milk samples were obtained from ten grazing dairy cows. SE = Standard error.

Milk fraction	Equation	R <sup>2</sup>	SE (Observation)	P value
Whole milk	Y = 0.951X + 0.51	0.74	0.14	<0.001
Milk protein	Y = 1.007X + 0.11	0.64	0.16	0.003
Milk non-protein N	Y = 1.019X – 0.55	0.81	0.17	<0.001

means in the study of Cheng and Dewhurst (2009). The  $\delta^{15}\text{N}$  of whole milk was close to that in milk protein, whilst the  $\delta^{15}\text{N}$  of the non-protein N fraction was much lower. The relative contents of  $^{15}\text{N}$  in protein, non-protein N and whole milk suggest that the milk protein fraction made up on average 4.4% of milk N, which is very close to the measured value of 4.3%. The small, but highly significant, difference in fractionation between morning and evening samples may be related to diurnal variation in the rate or composition of herbage intake (Cosgrove *et al.*, 2009). Nonetheless, the highly significant relationships between individual morning and evening values demonstrate the consistent and strong cow effect and suggest that this low-cost approach will be useful for studies to understand genetic control of N-use efficiency.

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