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Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low-quality pasture in late lactation

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1 **Nitrogen partitioning, energy use efficiency and isotopic**
2 **fractionation measurements from cows differing in genetic merit**
3 **fed low quality pasture in late lactation**

4

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13

14

15 **Summary text for the Table of Contents**

16

17 No information is available regarding nitrogen and energy use efficiency in relation to nitrogen

18 isotopic fractionation measured from cows with different genetic merit. Eight high and eight low

19 breeding worth cows were used to conduct a nitrogen balance study, and the results showed

20 nitrogen isotopic fractionation and breeding worth were useful indicators of dry matter intake and

21 nitrogen use efficiency of individual cows, respectively. Both indicators are easy to obtain on farm

22 and could be further developed for dairy cow breeding programmes.

23

24 **Abstract.** The study was carried out to evaluate energy and nitrogen (N) use efficiencies of high and
25 low breeding worth (BW) cow groups relative to N isotopic fractionation ($\Delta^{15}\text{N}$). Eight high and eight
26 low BW cows (mean BW index = 198 and 57, respectively) in late lactation were used to conduct an N
27 balance study with all cows fed autumn pasture. Individual cow pasture DM intake, N intake and N
28 outputs of milk, urine and faeces were quantified. Plasma sample from each cow was harvested.
29 Feed, plasma, faeces, urine and milk samples were measured for $\delta^{15}\text{N}$ and calculated for $\Delta^{15}\text{N}$. Urea
30 nitrogen in milk and plasma, and urinary excretion of purine derivatives were also measured. The
31 metabolisable energy (ME) intake, milk energy output, and energy and N use efficiencies of high BW
32 cows were greater on average than low BW cows. Conversely, the ratios of urinary N excretion to
33 faecal N excretion and urinary N excretion to N intake were greater for low BW cows than high BW
34 cows. There was no effect of BW groups on manure N output, apparent N digestibility, retained N,
35 purine derivatives excretion or ratio of purine derivatives excretion to ME intake. No relationships
36 were found between N and energy efficiencies and $\delta^{15}\text{N}$ measurements. Regression analysis with
37 individual cow measurement showed plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ was negatively correlated with DM
38 intake. N use efficiency was positively correlated with BW. High genetic merit cows are more efficient
39 in N and energy use than lower genetic merit cows when fed low quality pasture in late lactation.
40 Plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ was proved to be a potential indicator of DM intake for individual cows when
41 identical feed was offered. BW may be used to predict N use efficiency for individual cows.

42

43 **Keywords:** sustainability, isotopic discrimination, microbial protein synthesis, microbial energetic
44 efficiency, manure nitrogen

45

46 **Introduction**

47 The breeding objective of New Zealand dairy industry is to produce cows that are more efficient at
48 converting feed into profit. The genetic merit of a New Zealand dairy cow is measured by her
49 breeding worth (BW) index, which ranks a cow on her expected genetic ability to breed profitable

50 and efficient replacements. The BW ranking is derived from breeding values which are based on
51 ancestry, lactation performance and progeny information for seven traits (milk protein, milk fat, milk
52 yield, somatic cell, live weight, fertility, and residual survival – a measurement of longevity) and are
53 combined with specific economic values to derive a BW. In general, the BW system emphasises milk
54 fat and protein production with a negative weighting applied to milk volume (Berry *et al.* 2007).

55 Earlier research demonstrated that high genetic merit cows produce more milk than low
56 genetic merit cows when they are offered the same feed (Grainger *et al.* 1985; Coleman *et al.* 2010).
57 However, limited information is available regarding nitrogen (N) partitioning and nutrient use
58 efficiency of cows selected under the New Zealand BW system when fed pasture as a sole diet.

59 Previous research showed that N isotopic fractionation ($\delta^{15}\text{N}$) between plasma, milk and feed
60 can be used to reflect N use efficiency of cows fed on different protein sources (Cheng *et al.* 2013).
61 Further, plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ is also a promising predictor of feed conversion efficiency in growing
62 beef cattle reared on identical diets (Wheadon *et al.* 2014).

63 The objectives of this study were to 1. Compare energy and N use efficiencies of high and low
64 BW cows; and 2. Investigate the use of $\Delta^{15}\text{N}$ as a simple indicator of energy and N use efficiency.

65

66 **Materials and methods**

67 *Design and management*

68 The study was undertaken at DairyNZ Lye Farm, Hamilton, New Zealand under the authority of the
69 Ruakura Animal Ethics Committee. Sixteen multiparous Holstein-Friesian dairy cows in late lactation
70 (221 ± 22.0 days in milk and 549 ± 50.3 kg live weight (LW), mean \pm SD) were used, including eight
71 high BW (H, mean BW index = 198) and eight low BW (L, mean BW index = 57) cows. Cows grazed on
72 pasture prior to the study, and were then housed individually in metabolism stalls for four days to
73 adapt to the facilities prior to commencing a five-day N balance study. Cows were fed freshly cut
74 perennial ryegrass-based pasture at 0840 and 1600 h daily to ensure daily refusals were $\sim 10\%$ of

75 offered pasture (*ad libitum*). Live weight was recorded at the start and the end of the five-day
76 measurement period.

77

78 *Intake and pasture quality measurement*

79 Intake was measured daily per cow by weighing offered and refused pasture. Pasture samples (1 kg
80 fresh weight) were collected twice daily at feeding and oven-dried at 65°C before grinding through a
81 1.0-mm sieve (Christy Lab Mill; Suffolk; UK). Pasture samples were analysed by Near Infra-Red
82 Spectroscopy (NIRS systems 6500; feed TECH; New Zealand) for predicted crude protein (CP), organic
83 matter digestibility, acid detergent fibre, neutral detergent fibre, and soluble sugar and starch
84 content. Metabolisable Energy (ME) content was derived from predicted organic matter digestibility
85 on the basis of an *in vitro* cellulase digestibility assay (Roughan and Hollan 1977; Dowman and Collins
86 1982), calibrated against *in vivo* standards (Corson *et al.* 1999).

87

88 *Milk and plasma sampling and analysis*

89 Cows were milked twice daily at 0730 and 1530 h. Milk production was recorded (Tru-test milk
90 meters; Tru-test Ltd; New Zealand), and sub-sampled for composition analysis. A 10 ml blood sample
91 was collected using a Li-heparinized evacuated tube from the jugular vein of each cow at 1130 h
92 daily. Plasma was harvested following centrifugation at 1200 × *g* for 12 min at 4°C. At the end of the
93 collection period, milk and plasma samples were pooled per cow and stored at -20°C until analyses
94 were conducted.

95 Milk samples were analysed for fat, protein, and urea N (MUN) concentrations using Fourier-
96 Transform Infra-Red Spectroscopy (Fossomatic™; Foss Electric; Hillerød; Denmark). Milk N content
97 was derived from milk protein content divided by 6.38. Milk energy output (MJ/d) was calculated
98 according to Rattray *et al.* (2007): $[1.1 \times \text{milk production} \times (0.376 \times \text{milk fat \%} + 0.209 \times \text{milk protein}$
99 $\% + 0.976)]$. Plasma urea N (PUN) was quantified using a kinetic UV and colorimetric assay (Modular
100 P800; Germany). Freeze-dried samples of milk, plasma, and pasture were ground through a 1.0-mm

101 sieve and weighed before $\delta^{15}\text{N}$ analysis was conducted using an Isotope Ratio Mass Spectrometer
102 (PDZ Europa Ltd; UK; Cheng *et al.* 2011).

103

104 *Faeces and urine collection and nitrogen analysis*

105 Total outputs of faeces and urine from each cow were collected daily using the externally applied
106 separators described by Meier *et al.* (2008). Urine containers were sealed to minimise N volatilisation
107 losses. Daily urine samples were collected at 0730 and 1930 h, and acidified to pH < 4 using
108 hydrochloric acid to prevent N volatilisation before storage at -20°C . Faecal samples were freeze
109 dried before measurement. Representative liquid urine and freeze dried faeces from five days bulked
110 samples per cow were sub sampled and measured for N concentration using a Variomax CN Analyser
111 (Elementar Analysensysteme GmbH; Hanau; Germany). Retained N was calculated from N intake (NI)
112 and N outputs: NI - milk N (MN) - faecal N (FN) - urinary N (UN). Manure N output was calculated by
113 adding UN and FN together. Apparent N digestibility was calculated: $(\text{NI} - \text{FN}) \div \text{NI}$. Urinary excretion
114 of purine derivatives (PD) was analysed using HPLC (Agilent 1100 series; Germany) following the
115 method described by George *et al.* (2006).

116

117 *Efficiency calculations*

118 Energy use efficiency (EUE) = milk energy output (MJ/day) \div [DMI (kg/day) \times ME content of pasture
119 (MJ/kg DM)]

120 N use efficiency (NUE) = MN (g/day) \div NI (g/day)

121 Microbial energetic efficiency (MEE) = PD (mmol/day) \div [DMI (kg/day) \times ME content of pasture
122 (MJ/kg DM)]

123

124 *Statistical analysis*

125 The Genstat statistical package (version 15.1) was used for general analysis of variance and linear
126 regression analysis. The statistical model included the treatment effect of BW (H and L). Data from

127 the five measurement days were averaged for individual cows for each variable and means were
 128 analysed using ANOVA. The significance of treatment effect was declared at $P < 0.05$.

129

130 **Results**

131 The quality of pasture (Table 1) offered to cows in this study was typical for the autumn season in the
 132 Waikato region of New Zealand. The intakes of DM and ME were higher for H compared with L (Table
 133 2). Cows in the H group had 23% higher milk energy output and 15% higher feed energy utilisation
 134 for milk energy output than cows in L (Table 2). Energy intake explained 44% of the variation in milk
 135 energy output in the current study. NI and MN were 388 and 85, and 360 and 66 g/d for H and L,
 136 respectively. NUE was 22% higher for cows in H than cows in L. FN increased as NI increased ($r^2 =$
 137 52.8 , $SE = 8.74$, $P < 0.001$). In addition, UN: NI and UN: FN ratios were, respectively, 13% and 15%
 138 lower for H compared to L. However, there was no difference in manure N output between
 139 treatments (Table 2). The calculated apparent N digestibility did not differ between H (0.67 g/g) and L
 140 (0.68 g/g). Estimated retained N was 20.2 g/cow.d and 12.1 g/cow.d for H and L respectively, with no
 141 statistical difference determined.

142 **[Insert Table1 and 2 here]**

143 N isotopic fractionation and urea N content in milk and plasma did not differ between H and
 144 L groups. No statistical difference was detected for PD and MEE (Table 2).

145 Using individual cow measurements, significant positive relationships were found between
 146 MUN and PUN [$PUN \text{ (mmol/l)} = -0.36 + 0.73 \times MUN \text{ (mmol/l)}$; $r^2 = 34.1$, $SE = 0.412$, $P < 0.05$], and milk
 147 $\delta^{15}N - \text{feed } \delta^{15}N$ and plasma $\delta^{15}N - \text{feed } \delta^{15}N$ [$\text{plasma } \delta^{15}N - \text{feed } \delta^{15}N \text{ (‰)} = 1.95 + 0.53 \times \text{milk } \delta^{15}N -$
 148 $\text{feed } \delta^{15}N \text{ (‰)}$; and $r^2 = 24.8$, $SE = 0.254$, $P < 0.05$]. However, no relationship was found between
 149 nutrient use efficiencies and urea N in plasma and milk, and N isotopic fractionation of milk, plasma,
 150 urine and faeces. Although no correlation was found between individual cow measurements for
 151 plasma $\delta^{15}N - \text{feed } \delta^{15}N$ and NUE, and BW and DMI; plasma $\delta^{15}N - \text{feed } \delta^{15}N$ was significantly
 152 correlated with DMI (Fig 1b), and BW and NUE was also correlated (Fig 1a).

153 **[Insert Fig1 here]**

154

155 **Discussion**

156 *Intake and milk energy output*

157 The higher DMI and energy intake observed in H compared with L (Table 2) is consistent with
158 Coleman *et al.* (2010). Milk energy output proportionally increased as energy intake increased, this
159 indicates energy intake is one of the major drivers for milk energy output in cows differing in genetic
160 merit.

161

162 *Energy use efficiency*

163 There are five potential **factors** that might contribute to the difference in EUE between H and L
164 groups: 1) mobilisation of body reserves to support production; 2) preferential partitioning of ME
165 intake between milk and **body tissue**; 3) change in energy utilisation in the rumen; 4) change in the
166 efficiency of utilisation of ME for milk production (i.e. k_l); or 5) differences in maintenance ME
167 requirements (ME_m). The contribution from body reserves to production was not quantified in this
168 study, therefore this possibility cannot be excluded. However, that the cows were in late lactation
169 and were likely in positive energy balance (Bauman and Currie 1980) suggest that energy
170 mobilisation would have been minimal. The change of energy level in the body (i.e. loss or gain
171 energy) would be difficult to quantify during five day measurement period. However, Davey *et al.*
172 (1983) showed that high genetic merit cows were able to partition more energy intake to support
173 milk production, and this may be linked with growth hormone and blood metabolite (e.g. glucose)
174 differences between the cows. Rumen function was indicated by calculated PD and MEE; both
175 showed no difference between groups (Table 2). Ferris *et al.* (1999) reported that k_l was not affected
176 by cow genotype. Therefore, a value of $k_l = 0.6$ was adopted to calculate ME_m , but no statistical
177 difference was detected.

178

179 *Nitrogen use efficiency*

180 Because FN increased in proportion to NI in this study, there was no difference between the H and L
181 groups in apparent N digestibility. Similar to Ferris *et al.* (1999), the proportion of NI partitioned to
182 urine (UN : NI) was lower for cows in the H group than in the L group (Table 2). The lower UN : FN
183 ratio for H was due to lower UN and higher FN without altering rumen function in the current study.
184 This is in contrast to previous report from Miller *et al.* (2001), who found the change of UN : FN was
185 due to the change of rumen function. The partitioning of surplus N away from urine to faeces may
186 contribute to reduce nitrate leaching to ground water and nitrous oxide and ammonia emission to
187 the atmosphere (Varel *et al.* 1999). Higher NUE for H compared with L was reported by Ferris *et al.*
188 (1999) and Wheadon *et al.* (2013) when comparing cow differing in genetic merit in Northern Ireland
189 and New Zealand, respectively. The change in NUE in the current study was mainly achieved by 29%
190 higher MN in H than in L, rather than altering rumen microbial protein synthesis or deamination in
191 the liver (indicated by N isotopic fractionation) per se, which was suggested by Cheng *et al.* (2013). It
192 is important to note that Wheadon *et al.* (2013) also suggested that higher metabolisable protein
193 efficiency (i.e. milk protein output: metabolisable protein intake) for high genetic merit cows may be
194 a factor that contributes to a higher NUE.

195

196 *Relationships between urea and nitrogen use efficiency*

197 The positive linear relationship between MUN and PUN detected in the current study is consistent
198 with previous research (Hof *et al.* 1997; Kauffman and St-Pierre 2001). This relationship is due to
199 urea produced by the liver and carried by the blood to the kidney. Urea can freely diffuse from blood
200 to other body fluids, including milk (Kauffman and St-Pierre 2001). Although urea N in milk and
201 plasma were found to be good indicators of NUE when various feeds were offered (Broderick and
202 Clayton 1997), no relationship was found in the current study. The reason for this lack of relationship
203 may be related to little difference in rumen fermentation, as previous research suggested that 80%
204 of the variation in MUN is from rumen fermentation (Hof *et al.* 1997).

205

206 *Nitrogen isotopic fractionation in relation to nitrogen use efficiency and intake*

207 The levels of N isotopic fractionation presented in Table 2 are within the normal ranges previously
208 reported (Cheng *et al.* 2011; Wheadon *et al.* 2014). The weak but significant relationship between
209 milk $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ and plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ is in agreement with Cheng *et al.* (2013). The effect
210 may be related to N isotopic fractionation during digestion and absorption or a common effect of the
211 endogenous contribution to the N sinks in the body.

212 In contrast to Cheng *et al.* (2013), there was no significant relationship between plasma $\Delta^{15}\text{N}$
213 (plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$) and NUE in this study. The absence of any difference in PD and MEE
214 suggests rumen function was similar for cows in both groups. Therefore the higher milk protein
215 synthesis observed for cows in the H group was probably due to improved utilisation of un-
216 degradable dietary protein rather than alterations in the supply of rumen degradable protein. In
217 addition, it is important to note that the predicted values of plasma $\Delta^{15}\text{N}$ from NUE using the
218 equation from Cheng *et al.* (2013) were 3.53 and 3.99 for H and L, respectively. In comparisons to the
219 measured values for plasma $\Delta^{15}\text{N}$ of 3.81 and 4.02 for H and L (Table 2), the predicted values were
220 within typical levels of standard deviation for isotope measurement (i.e. 0.3‰). Therefore, the
221 absence of a relationship between plasma $\Delta^{15}\text{N}$ and NUE in this study may also be due to inability of
222 Isotope Ratio Mass Spectrometry to detect isotopic fractionation differences less than 0.3‰.

223 It is important to note that considerable variation exists in pasture $\delta^{15}\text{N}$ from the same farm,
224 partly due to the relative contribution of clover and grass to the mix pasture sward (Cheng,
225 unpublished data). Legumes fix $\delta^{15}\text{N}$ depleted nitrogen from the atmosphere and generally have a
226 lower $\delta^{15}\text{N}$ than grass (Steele and Daniel, 1978). Therefore, the pasture $\delta^{15}\text{N}$ each cow group
227 ingested may have differed. Although all cows were offered with same swards, it is possible that feed
228 $\delta^{15}\text{N}$ varied as a result of differences in feed selectivity between H and L cows. Future study is needed
229 to sample feed from each cow or each treatment group in order to provide more accurate data to
230 establish correlations.

231 The negative relationship between DMI and plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ (Fig 1b) was similar to
232 the result presented by Sick *et al.* (1997), who showed when N is lower than the requirement for
233 optimal growth of rats, transamination/deamination reactions drive a negative relationship between
234 NI and plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$. In the current study, the feed contained only 2.43% N which is less
235 than the requirement (i.e. 2.88% N) suggested for lactating cows by Pacheco and Waghorn (2008).
236 Kristensen *et al.* (2010) showed that the higher the NI, the lower the absolute amount of recycled
237 urea that would end up in the rumen. This should result in an enrichment of $\delta^{15}\text{N}$ in microbial protein
238 which contributes to the plasma protein pool (Wattiaux and Reed, 1995), and consequently lead to a
239 positive relationship between DMI and plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$. However, the contribution of rumen
240 N isotopic fractionation in this study would have been minimal, since no variation was found for
241 rumen function indicators (PD and MEE; Table 2).

242

243 *Breeding worth in relation to nitrogen use efficiency and intake*

244 The positive relationship found between NUE and BW (Fig 1a) indicates the current breeding
245 objectives for dairy cows (which place a high weighting on milk protein and fat production) will also
246 result in an improvement in NUE. This study also supports previous research showing that higher BW
247 cows have higher intakes (Table 2; Grainger *et al.* 1985).

248

249 **Conclusion**

250 Selection of cows for increased genetic merit for milk fat and protein production (as indicated by the
251 BW index) also leads to improved energy and N use efficiencies. Cows in the H group excreted a
252 lower proportion of their N intake in the urine than cows in the L group. Milk and plasma urea N
253 were not associated with NUE. In addition, this study demonstrated that N isotopic fractionation
254 between plasma and feed may be developed as an indicator of DMI for individual cows when
255 identical feed is offered.

256

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264

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337

Table 1. Chemical composition and $\delta^{15}\text{N}$ content of feed

338

Item	Pasture
DM (%)	21.0
Metabolisable energy (MJ/kg DM)	9.87
Crude protein (% on DM basis)	15.2
Neutral detergent fibre (% on DM basis)	58.9
Acid detergent fibre (% on DM basis)	31.8
Soluble sugars and starch (% on DM basis)	6.01
Feed $\delta^{15}\text{N}$ (‰)	2.97

339

340

341 **Table 2. Genetic merit, live weight, intake, milk composition, energy use efficiency, nitrogen (N)**
 342 **partitioning, N isotopic fractionation and biomarkers of high (H) and low (L) breeding worth cows.**

343

344	Item	H	L	SED	Significance
345	Genetic merit (breeding worth; \$)	198	57	9.8	-
346	Live weight change (kg/day)	1.0	0.6	1.14	NS
347	Dry matter intake (kg DM/cow.day)	16.0	14.8	0.48	*
348	Milk production (kg/cow.d)	13.6	12.4	0.49	*
349	Milk fat (%)	5.72	4.98	0.347	NS
350	Milk protein (%)	3.98	3.42	0.124	***
351	Energy intake (MJ ME/cow.day) ^A	157.8	146.4	4.76	*
352	Milk energy output (MJ/cow.day) ^B	59.1	48.3	2.14	***
353	Energy use efficiency (MJ/MJ) ^C	0.38	0.33	0.013	**
354					
355	N intake (g/cow.day)	387.9	359.7	11.72	*
356	Milk N (g/cow.day)	84.8	65.9	3.03	***
357	Faecal N (g/cow.day)	129.1	116.7	5.69	*
358	Urinary N (g/cow.day)	153.7	165.0	8.08	NS
359	Retained N (g/cow.day) ^D	20.2	12.1	9.00	NS
360	N use efficiency (g/g) ^E	0.22	0.18	0.009	**
361	Urinary N: N intake (g/g)	0.40	0.46	0.019	**
362	Urinary N : faecal N (g/g)	1.20	1.42	0.074	**
363	Manure N output (g/g) ^F	282.9	281.7	11.11	NS
364					
365	Milk $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ (‰)	3.61	3.83	0.140	NS
366	Plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ (‰)	3.81	4.02	0.137	NS

367	Urine $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ (‰)	-2.09	-1.93	0.231	NS
368	Faeces $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$ (‰)	1.48	1.66	0.309	NS
369	Milk urea N (mmol/l)	6.88	7.19	0.250	NS
370	Plasma urea N (mmol/l)	4.62	4.94	0.297	NS
371	Purine derivatives (mmol/cow.day) ^G	148.6	160.5	11.63	NS
372	Purine derivatives : ME intake				
373	(mmol/MJ) ^H	0.94	1.11	0.077	NS

374 ^A dry matter intake \times pasture metabolisable energy content

375 ^B $[1.1 \times \text{milk production} \times (0.376 \times \text{milk fat \%} + 0.209 \times \text{milk protein \%} + 0.976)]$ according to Rattray

376 *et al.* (2007)

377 ^C milk energy output : ME intake

378 ^D N intake - faecal N - urinary N - milk N

379 ^E milk N : N intake

380 ^F urinary N + faecal N

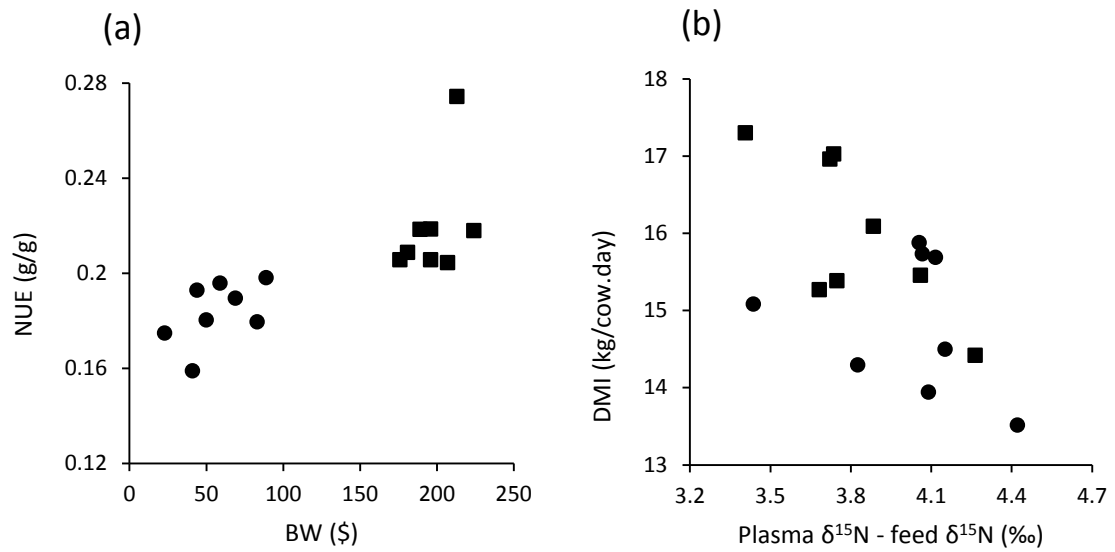
381 ^G indicator of microbial protein synthesis

382 ^H indicator of microbial energetic efficiency

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385



386

387 **Fig. 1.** Relationship between breeding worth (BW) and nitrogen use efficiency (NUE) (a), plasma $\delta^{15}\text{N}$
 388 - feed $\delta^{15}\text{N}$ and dry matter intake (DMI) (b) of high (■) and low (●) genetic merit cows fed on autumn
 389 pasture in late lactation.

390

391 Fig 1a: $\text{NUE (g/g)} = 0.17 + 0.0003 \times \text{BW (\$)}$

392 ($n = 16$, $r^2 = 55.4$, $\text{SE} = 0.017$, $P < 0.001$)

393 Fig 1b : $\text{DMI (kg/cow.d)} = 24.7 - 2.37 \times \text{plasma } \delta^{15}\text{N} - \text{feed } \delta^{15}\text{N (‰)}$

394 ($n = 16$, $r^2 = 32.5$, $\text{SE} = 0.909$, $P < 0.05$)

395