**Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: A meta-analysis**

G. Cantalapiedra-Hijar *et al.*

**Table S1**. Description of the experimental studies included in the database in terms of type of ruminant, number of animals, diets and periods, as well as basic descriptive statistics for N use efficiency (NUE; dependent variable) and N isotopic fractionation (Δ15Nanimal-diet; independent variable) analyzed in plasma (P) or milk proteins (M)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Animal | FL1, % | Diets | Periods |  | NUE, g/g | | | |  | Δ15Nanimal-diet, ‰ | | | | | |
|  | N2 |  | n | n |  | n | Mean±SD | Min | Max |  | n | |  | Mean3±SD | | | |
| Publication and study |  |  |  |  |  |  |  |  |  |  | P | M |  | P | | M | |
| Cantalapiedra-Hijar *et al.*, 2015 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No.1 (ID#1) | Growing beef cattle (36) | 1.66 | 4 | 1 |  | 34 | 0.235±0.045 | 0.152 | 0.324 |  | 34 |  |  | 3.56±0.39 |  | | |
| Study No.2 (ID#2) | Dairy cows (5) | 4.03 | 4 | 4 |  | 18 | 0.320±0.033 | 0.265 | 0.392 |  | 18 | 18 |  | 2.60±0.44a | | 2.18±0.39b | |
| Cantalapiedra-Hijar *et al.*, 2016 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No.3 (ID#3) | Dairy cows (16) | 3.04 | 4 | 4 |  | 16 | 0.270±0.032 | 0.217 | 0.320 |  | 16 |  |  | 2.41±0.38 | |  | |
| Cabrita *et al.*, 2014 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No.4 (ID#4) | Dairy cows (9) | 3.62 | 3 | 3 |  | 24 | 0.296±0.042 | 0.232 | 0.394 |  |  | 24 |  |  | | 2.15±0.66 | |
| Study No.5 (ID#5) | Dairy cows (9) | 4.37 | 3 | 3 |  | 25 | 0.295±0.031 | 0.239 | 0.368 |  |  | 25 |  |  | | 2.45±0.37 | |
| Study No.6 (ID#6) | Dairy cows (9) | 3.76 | 3 | 3 |  | 20 | 0.341±0.031 | 0.294 | 0.397 |  |  | 20 |  |  | | 1.01±0.54 | |
| Cheng *et al.*, 2011 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No. 7 (ID#7) | Dairy cows (9) | 2.99 | 6\* | 3 |  | 18\* | 0.261±0.053 | 0.177 | 0.347 |  |  | 18\* |  |  | | 3.17±0.75 | |
| Cheng *et al.*, 2013a |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No. 8 (ID#8) | Non-lactating sheep (6) | 2.12 | 3 | 3 |  | 15 | 0.011±0.147 | -0.140 | 0.175 |  | 15 |  |  | 5.70±0.43 | |  | |
| Cheng *et al.*, 2013b |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No. 9 (ID#9) | Dairy cows (15) | 3.49 | 3 | 1 |  | 15 | 0.207±0.040 | 0.149 | 0.279 |  | 15 | 15 |  | 3.76±0.36a | | 3.68±0.44b | |
| Cheng *et al.*, 2014 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No. 10 (ID#10) | Dairy cows (16) | 2.80 | 1 | 1 |  | 16 | 0.201±0.026 | 0.159 | 0.274 |  | 16 | 16 |  | 3.72±0.29a | | 3.92±0.28b | |
| Cheng *et al.*, 2016 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | |
| Study No. 11 (ID#11) | Dairy goats (8) | 4.02 | 4 | 3 |  | 16 | 0.131±0.018 | 0.102 | 0.164 |  | 16 | 16 |  | 5.37±0.79 | | 5.35±0.77 | |
| Total |  |  | 38 |  |  | 217 | 0.243±0.095 | -0.140 | 0.392 |  | 130 | 152 |  | 3.28±1.34 | | | |

1 Feeding level (100 × kg dry matter intake/kg body weight)

2 n= Number of observations/data point

3 No differences (P> 0.05) were observed between Δ15Nanimal-diet in plasma vs milk when only studies using both matrices were analyzed (n = 130)

a,b Means in a row with different superscripts are significantly different (Test student; *P* < 0.05).

\* Three diets out of 9 were removed from the database because of their high ammonia-N content (see discussion).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | CP  g/kg DM | Net Energy  Mcal/kg DM | RDP  g/kg DM | RPB  g/kg DM | EMPS\_E  g/kg fOM | EMPS\_N  g/kg RDP | DENU  g/g | EMPU\_prod  g/g | EMPU\_tot  g/g |
| Publication and study |  |  |  |  |  |  |  |  |  |  |
| Cantalapiedra-Hijar *et al.*, 2015 |  |  |  |  |  |  |  |  |  |  |
| Study No.1 (ID#1) |  | 162±3.15 | 1.65±0.028 | 125±1.75 | 25.4±0.88 | 152±6.93 | 682±9.85 | 0.524±0.014 | 0.393±0.057 | 0.733±0.094 |
| Study No.2 (ID#2) |  | 148±28.2 | 1.57±0.024 | 101±17 | 4.25±18 | 184±14 | 836±158 | 0.625±0.059 | 0.472±0.021 | 0.731±0.029 |
| Cantalapiedra-Hijar *et al.*, 2016 |  |  |  |  |  |  |  |  |  |  |
| Study No.3 (ID#3) |  | 128±18.9 | 1.16±0.035 | 84.0±12.4 | -10.5±13 | 180±4.15 | 973±152 | 0.683±0.056 | 0.392±0.016 | 0.682±0.036 |
| Cabrita *et al.*, 2014 |  |  |  |  |  |  |  |  |  |  |
| Study No.4 (ID#4) |  | 157±18.1 | 1.64±0.022 | 111±18 | 20.0±21 | 172±2.65 | 707±153 | 0.587±0.083 | 0.479±0.016 | 0.718±0.032 |
| Study No.5 (ID#5) |  | 170±4.36 | 1.72±0.019 | 117±3.79 | 25.4±3.25 | 173±1.77 | 661±17 | 0.578±0.021 | 0.480±0.009 | 0.690±0.020 |
| Study No.6 (ID#6)2 |  | 145 | 1.63 | 110 | 18.6 | 174 | 701 | 0.573 | 0.535 | 0.796 |
| Cheng *et al.*, 2011 |  |  |  |  |  |  |  |  |  |  |
| Study No. 7 (ID#7) |  | 163±27.0 | 1.55±0.098 | 118±20 | 30.0±22 | 160±6.99 | 640±107 | 0.488±0.055 | 0.518±0.012 | 0.857±0.028 |
| Cheng *et al.*, 2013a |  |  |  |  |  |  |  |  |  |  |
| Study No. 8 (ID#8) |  | 174±27.2 | 1.14±0.084 | 121±30 | 33.2±29 | 169±17 | 629±134 | 0.492±0.082 | 0.065±0.067 | 0.402±0.035 |
| Cheng *et al.*, 2013b |  |  |  |  |  |  |  |  |  |  |
| Study No. 9 (ID#9) |  | 199±30.4 | 1.53±0.014 | 151±33 | 58.3±32 | 147±1.78 | 535±118 | 0.463±0.081 | 0.455±0.026 | 0.707±0.017 |
| Cheng *et al.*, 2014 |  |  |  |  |  |  |  |  |  |  |
| Study No. 10 (ID#10)3 |  | 152 | 1.55 | 110 | 14.9 | 147 | 735 | 0.569 | 0.352 | 0.631 |
| Cheng *et al.*, 2016 |  |  |  |  |  |  |  |  |  |  |
| Study No. 11 (ID#11) |  | 268±4.97 | 1.99±0.117 | 183±5.02 | 85.4±1.98 | 147±5.85 | 457±4.89 | 0.421 | 0.321±0.033 | 0.499±0.032 |

**Table S2**. Description of the experimental diets included in the database in terms of crude protein and net energy contents and parameters related to N utilization by ruminants.

1 RDP = Rumen degradable protein; RPB = Rumen protein balance; EMPS\_E = Efficiency of microbial protein synthesis according to the available energy; EMPS\_N = Efficiency of microbial protein synthesis according to rumen degradable protein; DENU = Digestive efficiency N use; EMPU\_prod = Efficiency of metabolizable protein use for production; EMPU\_tot = Efficiency of metabolizable protein use for total net protein synthesis

2 The three treatments from experiment 3 in Cabrita *et al.* (2014) were reduced to 1 diet because the difference between them was only due to the feeding pattern.

3 The two experimental conditions from Cheng *et al.* (2014) corresponded to two different animal selection lines (low vs high efficiency animals) and used the same diet



**Figure S1**. Relationship between N use efficiency and N isotopic fractionation (Δ15Nanimal-diet) in ruminants using individual values (n = 282). a) Simple linear regression analysis (overall relationship: *NUE = 0.429 – 0.058 × Δ15Nanimal-diet*) showing the distribution of individual values according to the type of sample (open circles = milk, closed circles = plasma), b) Simple linear regression analysis using only studies (n = 4) reporting the 15N natural abundance both in milk and plasma samples. Open circles = milk (*NUE = 0.425 − 0.055 × Δ15Nanimal-diet*), closed circles = plasma (*NUE = 0.446 − 0.059 × Δ15Nanimal-diet)*. No effect of sample type was noted either on the intercept (*P = 0.28*) or on the slope (*P = 0.39*).