

Scotland's Rural College

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

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

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20

21 Short title: Prediction of nitrogen use efficiency in ruminants

22 **Abstract:**

23 Animal proteins are naturally  $^{15}\text{N}$  enriched relative to the diet and the extent of this  
24 difference ( $\Delta^{15}\text{N}_{\text{animal-diet}}$  or N isotopic fractionation) has been correlated to N use  
25 efficiency (**NUE**; nitrogen gain or milk N yield/N intake) in some recent ruminant  
26 studies. The present study used meta-analysis to investigate whether  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
27 can be used as a predictor of NUE across a range of dietary conditions, particularly  
28 at the level of between-animal variation. An additional objective was to identify  
29 variables related to N partitioning explaining the link between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$ .  
30 Individual values from 8 publications reporting both NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  for  
31 domestic ruminants were used to create a database comprising 11 experimental  
32 studies, 41 treatments and individual animal values for NUE (n = 226) and  $\Delta^{15}\text{N}_{\text{animal-}}$   
33  $\text{diet}$  (n = 291). Data were analyzed by mixed-effect regression analysis taking into  
34 account experimental factors as random effects on both the intercept and slope of  
35 the model. Diets were characterized according to the INRA feeding system in terms  
36 of N utilization at the rumen, digestive and metabolic levels. These variables were  
37 used in a Partial Least Squares regression analysis to predict separately NUE and  
38  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation, with the objective of identifying common variables linking NUE  
39 and  $\Delta^{15}\text{N}_{\text{animal-diet}}$ . For individuals reared under similar conditions (within-study) and at  
40 the same time (within-period), the variance of NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  not explained by  
41 dietary treatments (i.e. between-animal variation plus experimental error) was 35%  
42 and 55% respectively. Mixed-effect regression analysis conducted with treatment  
43 means showed that  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was significantly and negatively correlated to NUE  
44 variation across diets ( $\text{NUE} = 0.415 - 0.055 \times \Delta^{15}\text{N}_{\text{animal-diet}}$ ). When using individual  
45 values and taking into account the random effects of study, period and diet, the  
46 relationship was also significant ( $\text{NUE} = 0.358 - 0.035 \times \Delta^{15}\text{N}_{\text{animal-diet}}$ ). However, there

47 may be a biased prediction for animals close to zero, or in negative, N balance.  
48 When using a novel statistical approach, attempting to regress between-animal  
49 variation in NUE on between-animal variation in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  (without the influence of  
50 experimental factors), the negative relationship was still significant, highlighting the  
51 ability of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  to capture individual variability. Among the studied variables  
52 related to N utilization, those concerning N efficiency use at the metabolic level  
53 contributed most to predict both  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE variation, with rumen  
54 fermentation and digestion contributing to a lesser extent. This study confirmed that  
55 on average  $\Delta^{15}\text{N}_{\text{animal-diet}}$  can predict NUE variation across diets and across  
56 individuals reared under similar conditions.

57

58 **Keywords:**  $^{15}\text{N}$ , ruminant, nitrogen use efficiency, meta-analysis

59

60 **Implications:**

61 Variation in the N use efficiency in ruminants across diets, but also across  
62 individuals, can be predicted from the difference in the natural abundance of  $^{15}\text{N}$   
63 between the animal proteins and diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ). The ability of this isotopic  
64 biomarker to rank individuals from a homogenous group (same animal species,  
65 physiological status and fed the same diet at the same time) could open the door to  
66 its application in genetic selection programs and precision livestock feeding.

67

68 **Introduction**

69 Improving the conversion of feed resources into animal products should be a goal in  
70 animal production systems aiming to solve future food security issues. Identifying  
71 feeding strategies, but also individual animals, leading to a greater efficiency of

72 nutrient utilization is therefore of crucial importance. Nitrogen use efficiency (**NUE**;  
73 nitrogen gain or milk N yield/N intake) is an important component of ruminant feed  
74 efficiency (Wheadon *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015) and logically  
75 determines the extent of N excretion to the environment. However, determination of  
76 NUE remains costly, laborious and difficult to accomplish under practical conditions.  
77 Accurate predictors of NUE are needed for precision management (feeding to  
78 individual potential), as well as for genetic selection. Previous studies have  
79 concluded that milk-N urea concentration in bulk tank milk (Kauffman and St-Pierre,  
80 2001) or an average blood urea-N concentration (Kohn *et al.*, 2005) may be useful  
81 indicators of on-farm NUE. However, they appear less suitable for monitoring protein  
82 nutrition and N utilization of individual animals (Hof *et al.*, 1997; Huhtanen *et al.*,  
83 2015), nor as a phenotyping tool to be applied in genetic selection for improved NUE  
84 (Vallimont *et al.*, 2010). An alternative and new biomarker for NUE in ruminants is  
85 based on the N isotopic fractionation between the animal and its diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ,  
86 for details see Cantalapiedra-Hijar *et al.*, 2016). Variation in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  has a high  
87 potential to discriminate individuals fed the same diet but showing different N  
88 partitioning, as suggested by studies carried out in different animal species (Gaye-  
89 Siessegger *et al.*, 2004; Sears *et al.*, 2009; Warinner and Tuross, 2010) and humans  
90 (Fuller *et al.*, 2014). Several recent studies have evaluated this new biomarker of  
91 NUE in ruminants with promising results in most, although not all, cases (Cheng *et al.*  
92 *et al.*, 2013; Cabrita *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015). The generalization of  
93 the relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  across different experimental  
94 conditions in ruminants has not yet been explored, nor has the potential of this new  
95 isotopic biomarker to predict between-animal variation in NUE.

96           Because ruminant N losses occur at both the rumen and animal metabolism  
97 levels (Dijkstra *et al.*, 2013), accurate biomarkers of NUE should be able to describe  
98 N partitioning at both levels. Although N isotopic fractionation in ruminants has been  
99 linked to both rumen bacterial (Sutoh *et al.*, 1993; Wattiaux and Reed, 1995) and  
100 splanchnic tissue (Cantalapiedra-Hijar *et al.*, 2015) metabolism, the significance of  
101 these pathways and biological mechanisms has only been evaluated under specific  
102 feeding conditions and with few animals (Cantalapiedra-Hijar *et al.*, 2016). Therefore,  
103 this study used meta-analysis to gauge the extent to which this isotopic biomarker  
104 can be proposed as a generalizable predictor of NUE variation – particularly at the  
105 level of between-animal variation (NUE variation in animals reared under identical  
106 conditions). An additional objective was to evaluate at which level (rumen, total  
107 gastrointestinal tract or metabolism) the N partitioning and utilization had a higher  
108 impact on the relationship between  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE in ruminant animals under  
109 a range of feeding conditions.

110

## 111 **Material and methods**

### 112 *Experimental data*

113 Individual values from all available publications (Cheng *et al.*, 2011, 2013a, 2013b,  
114 2014, 2016; Cabrita *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015, 2016) reporting  
115 both NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values in domestic ruminants were used to create a  
116 database ( $n_{\text{study}} = 8$ ). Overall, the database comprises 11 experimental studies (ID#1  
117 to ID#11), 41 treatments (ID#\_T) and individual animal values for NUE ( $n_{\text{indiv}} = 226$ )  
118 and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  ( $n_{\text{milk}} = 161$  and  $n_{\text{plasma}} = 130$ ) measured in dairy cattle ( $n_{\text{study}} = 7$ ),  
119 beef cattle ( $n_{\text{study}} = 2$ ), dairy goats ( $n_{\text{study}} = 1$ ) and non-lactating sheep ( $n_{\text{study}} = 1$ ).  
120 Trials were conducted as either factorial ( $n_{\text{study}} = 3$ ) or Latin square-like design

121 ( $n_{\text{study}} = 8$ ). A description of the experimental studies and diets used in our database  
122 is available in the Supplementary material (Supplementary Tables S1 and S2).

123

#### 124 *Diet characterization and N utilization assessment*

125 To evaluate at which level (rumen, total gastrointestinal tract or metabolism) the N  
126 utilization had a higher impact on the relationship between  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE,  
127 experimental diets were characterized according to the new updated INRA feeding  
128 system (Sauvant and Nozière, 2016) and always using treatment mean values.  
129 Ingredients and the chemical composition of diets, average feed intake and average  
130 animal body weight from each dietary treatment were used to determine theoretical  
131 feed values, as well as theoretical digestive and metabolic variables related to N  
132 utilization through the systool software ([www.systool.fr](http://www.systool.fr)). The relationship between  
133 theoretical and measured dietary compositions did not differ from the first bisector  
134 with RMSE < 20 and 50 g/kg DM for CP and NDF, respectively. Diets were thus  
135 described in terms of rumen protein balance (**RPB**, g/kg DM), rumen degradable  
136 protein (**RDP**, g/kg DM), efficiency of microbial protein synthesis according to either  
137 available energy (**EMPS\_E**, g/g rumen fermentable OM) or available protein  
138 (**EMPS\_N**, g /g RDP), the digestive efficiency of N use (**DENU**, g metabolizable  
139 protein [PDI in the French system]/g crude protein) and the efficiency of MP utilization  
140 for either production (**EMPU\_prod**, g of milk N secretion or N retention/g N from  
141 metabolizable protein intake) or for total net protein synthesis (**EMPU\_tot**, [g of milk  
142 N secretion or N retention + endogenous fecal N + N lost in scurf]/[g N from  
143 metabolizable protein intake – g endogenous urinary N excretion]). These new  
144 variables were added to our database.

145

146 *Statistical analysis*

147 *Sources of variation in NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$*

148 We fitted a random intercept model, through the nlme package in the R  
149 software (R Development Core Team, 2015), describing variability in NUE and  
150  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , separately:

151

152 **(Eq. 1)**  $Y_{ij} = \beta_0 + \beta_i + \varepsilon_{ij}$ ,

153

154 where,  $Y_{ij}$  is the observed NUE or  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values for observation  $j$  on the group  $i$ ,  
155  $\beta_0$  is the mean value across the population of domestic ruminants being sampled,  $\beta_i$   
156 is a random variable representing the deviation from the population mean for the  $i^{\text{th}}$   
157 group, and  $\varepsilon_{ij}$  is a random variable representing the deviation in NUE or  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
158 values for observation  $j$  on group  $i$  from the mean value for group  $i$ . The grouping  
159 factor (ID/P/D) included multiple nested levels of random effects for diet (D) within  
160 experimental period (P) within study (ID). The residual error of this model  
161 represented the between-animal variability together with the experimental error. Total  
162 variance explained by the grouping factor was split to assess the proportion of  
163 variance explained by each source of variation (ID, ID/P and ID/P/D).

164 Several parameters of interest were estimated with this approach, namely the  
165 average values for NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  for an “average” ruminant ( $\beta_0$ ), the variance  
166 of NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  among experimental conditions (*between-group variability*  
167 [ $\sigma_i^2$ ]), and the variances of NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  within the experimental unit (*within-*  
168 *group variability* [ $\sigma_e^2$ ]). With this random intercept model the intra-class correlation  
169 coefficient (**ICC**) was calculated to assess the explained variance due to each level of  
170 the nested grouping factor included in the model (ID, ID/P and ID/P/D). The ICC

171 gives an idea of the importance of the random variable (study, period and diet) to  
172 explain variation in NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values and it identified which grouping  
173 factors should be considered in the final mixed-effect model. The ICC was calculated  
174 as:

$$175 \text{ ICC} = \sigma_i^2 / (\sigma_i^2 + \sigma_e^2)$$

176

### 177 *Analysis of the relationship between NUE and $\Delta^{15}\text{N}_{\text{animal-diet}}$*

178 First, we used the `lmList` function of the `nlme` package (Pinheiro and Bates,  
179 2000) to fit linear regressions relating NUE to  $\Delta^{15}\text{N}_{\text{animal-diet}}$  for each study and dietary  
180 treatment separately (Figure 1). The confidence intervals were calculated for the  
181 individual regression coefficients and graphically presented (Figure 2) for evaluation  
182 of between-group variation. When there were indications of large study-to-study or  
183 diet-to-diet variation in either the intercept or slope estimates, a random effect was  
184 proposed in the model (Pinheiro and Bates, 2000).

185 Mixed-effect models were then used to test the ability of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  to reflect  
186 average NUE variation across diets and individuals. Different random structures  
187 (from simple to more complex models), were compared based on the Akaike  
188 Information Criterion (**AIC**; the lowest being best) and the Bayesian Information  
189 Criterion (**BIC**; the lowest being best). The random effects were tested on the  
190 intercept, slope or both. When information criterion statistics (AIC, BIC) were similar  
191 for two models, the log-likelihood ratio test criteria performed with the command  
192 ANOVA (model 0, model 1) in R was used to determine the best model. Random-  
193 effect structures were always compared (AIC, BIC) using the maximum likelihood  
194 method, while the coefficients of the final model were estimated using the restricted-  
195 maximum likelihood method.

196 The general form of the mixed-effect model was:

197 **(Eq. 2)**  $Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_{ij} + \varepsilon_{ij}$ ,

198

199 where  $Y_{ij}$  and  $X_{ij}$  are NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values, respectively,  $\beta_0$  and  $\beta_1$  are the  
200 fixed effects for the intercept and the slope, respectively; the  $b_i$  are the random-  
201 effects of experimental factors (the effect of study when using mean treatment values  
202 and the effect of either study, period within-study or diet within-period and study when  
203 using individual values) and assumed to be independent for different factors; and  $\varepsilon_{ij}$ ,  
204 are the identically distributed within-groups errors, assumed to be independent of the  
205 random effects. Because of the relative small number of data and complex random-  
206 effect structure, a diagonal variance-covariance structure for the random effects was  
207 chosen to obtain convergence for the most complex models (Pinheiro and Bates,  
208 2000).

209 An alternative approach, inspired from Phuong *et al.* (2013), was adopted to  
210 attempt to regress between-animal variation in NUE against between-animal variation  
211 in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  regardless of the influences of experimental factors. The between-  
212 animal variability in both NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was approached once the random  
213 effect of the experimental study (ID), period within-study (ID/P) and diet within-period  
214 and study (ID/P/D]) were removed from raw values according to Eq1. The resulting  
215 residuals for NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  (Table 1) were considered mainly due to the  
216 between-animal variation and unidentified sources of error (within-animal variation). If  
217 a relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was still significant once the raw values  
218 were devoid of the influence of experimental factors, the ability of this biomarker to  
219 capture between-animal variation in NUE would be proven.

220

## 221 *Identification of outliers*

222 A two-sided outlier test for the standardized residuals was performed at every  
223 step to identify outlier observations. Observations with absolute standardized  
224 residuals (random effects) greater than the  $1-(0.05/2)$  quantile of the standard  
225 normal distribution were thus identified (Pinheiro and Bates, 2000). Outliers were only  
226 removed from the database if biological reasons justified their elimination.

227

## 228 *Partial Least Square regression analysis*

229 To answer the question about which variables related to N utilization could  
230 better explain the link between  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE, a partial least-squares (**PLS**)  
231 regression analysis was carried out (XLStat v2015.2.02), independently for both  
232 variables, on descriptors of N use at the rumen, digestive and metabolic levels. PLS  
233 analysis circumvents the problem of multicollinearity between variables related to N  
234 partitioning. Thus, the PLS model included either NUE or  $\Delta^{15}\text{N}_{\text{animal-diet}}$  as the  
235 dependent variable and the 7 descriptors of N partitioning as independent variables  
236 (RPB, RPD, EMPS\_E, EMPS\_N, DENU, EMPU\_prod and EMPU\_tot). To determine  
237 the number of components to keep in the PLS model, the cross-validation criterion  
238 Q2 was considered ( $Q2 > 0.0975$ ). The importance of each variable in the model was  
239 assessed through their variable importance in projection (VIP) scores. Variables with  
240 VIP scores higher than one are considered most important.

241

## 242 **Results**

### 243 *Description of the meta-design*

244 Three diets out of 41 (nine individual NUE observations out of 226 [4.0%]) belonging  
245 to the same study (ID#7) were removed from our database based on both statistical

246 (large standardized residuals) and biological reasons as discussed later. The number  
247 of individual observations within each study ranged from 15 to 34 (Supplementary  
248 Table S1) and there were from 3 to 16 observations for each dietary treatment. Most  
249 studies (8 out of 11) tested the dietary treatments across several experimental  
250 periods (either 3 or 4) and in these cases each diet was tested on either 1 (ID#2,  
251 ID#3, ID#7) or 2 (ID#8 and ID#11) or 3 (ID#4, ID#5 and ID#6) animals per period. As  
252 shown in Supplementary Table S1, the 11 experiments cover a large range of NUE  
253 values (from -0.140 to 0.394 g/g) and show a high variability (CV = 40%) in relation to  
254 its mean value (0.243 g/g). This variability in NUE resulted from the heterogeneity of  
255 experimental studies in terms of type of ruminant, experimental diets (Supplementary  
256 Table S2; CP content ranging from 128 to 268 g/kg DM and NE content from 1.16 to  
257 1.99 Mcal/kg DM) and feeding level (Supplementary Table S2). The difference in  
258 natural  $^{15}\text{N}$  abundance between the ruminant and its diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) averaged  
259 3.28‰ (CV = 41%) and ranged from 1.01 to 5.70‰ across diets and studies. Four  
260 studies reported  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values both in plasma and milk proteins, but no effect  
261 of the type of sample (plasma vs milk) was noted either on the intercept ( $P = 0.28$ ) or  
262 on the slope ( $P = 0.39$ ) from the overall relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
263 across these four studies (Supplementary Figure S1). Thus, all  $\Delta^{15}\text{N}_{\text{animal-diet}}$  data  
264 ( $n_{\text{milk}} = 152$  and  $n_{\text{plasma}} = 130$ ) were used in subsequent regression analysis to  
265 improve model predictions.

266

### 267 *Identified sources of variation*

268 Variance components estimates (Table 1) calculated through a random  
269 intercept model showed the effect of study as a strong grouping factor explaining  
270 76% and 85% of the total variance (intra-class correlation coefficients) of NUE and

271  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , respectively. This means that around three quarters of variation  
272 observed in values for our dependent variable (NUE) was explained by variation  
273 among studies (between-study variability). The random effect of period (time) further  
274 explained one third of the remaining NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation. For this part,  
275 within a given study and experimental period the random effect of the diet explained  
276 around 65% and 45% of NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation, respectively.

277

### 278 *Relationship between NUE and $\Delta^{15}\text{N}_{\text{animal-diet}}$*

279 Figure 1 shows the relationship between NUE and N isotopic fractionation  
280 when all individual data ( $n_{\text{indiv}} = 217$ ) were regressed using a simple linear  
281 regression (between-study regression in Fig. 1a), or when individual linear fits were  
282 obtained for each study (within-study regression in Fig. 1b;  $n_{\text{study}} = 11$ ) or diet  
283 (within-diet regression in Fig. 1c;  $n_{\text{trt}} = 38$ ). A significant ( $P < 0.001$ ) quadratic term  
284 for  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was noted when conducting the between-study regression analysis  
285 (Fig. 1a), slightly improving the fit ( $R^2 = 0.75$ ; data not shown).

286 Although the response of NUE to  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation (slope) was always  
287 negative within-study (Figure 2a) only 6 out of 11 were significantly ( $P < 0.05$ )  
288 different from 0. Likewise, although most (31 out of 38) slopes were negative within-  
289 diet (Figure 2b) only around 29% (9 out of 31) were significantly ( $P < 0.05$ ) different  
290 from 0. The confidence intervals for the intercepts and slopes across studies and  
291 diets did not always overlap; with the most negative slope and highest intercept for  
292 the only study conducted with non-productive animals (study ID#8). A high variability  
293 among studies and diets was thus evidenced, suggesting the need for different  
294 intercepts and response (slope) coefficients among experimental conditions in our  
295 model.

296           Regardless of the approach (treatment means vs individual values) and based  
297 on AIC/BIC criteria, as well as on the comparison of random variance structure  
298 through the likelihood ratio test, the best mixed-model included the random effects of  
299 experimental factors on both the intercept and slope (Table 2); that is the most  
300 complex model structure. Based on variance component estimates of the best mixed-  
301 effect model using individual values, it is concluded that the influence of experimental  
302 factors (study, period and diet) was higher for variation in NUE response to  $\Delta^{15}\text{N}_{\text{animal-}}$   
303  $\text{diet}$  ( $\sigma = 0.010, 0.005$  and  $0.007$  for an average slope of  $-0.035$  [ $14\% < \text{CV} < 29\%$ ]) than  
304 for mean estimates of NUE ( $\sigma = 0.024, 0.0000019, 0.020$  for an average intercept of  
305  $0.358$  [ $\text{CV} < 7\%$ ]).

306           On average, the relationship between individual values of NUE and  $\Delta^{15}\text{N}_{\text{animal-}}$   
307  $\text{diet}$  adjusted by the random effects of the study, period (within-study) and diet (within-  
308 period and study) had a significant and negative slope of  $-0.035$  g/g (Figure 3) - much  
309 less pronounced than that obtained for the unadjusted between-study regression  
310 analysis (Figure 1;  $-0.058$  g/g) or when the analysis was based on treatment means  
311 and corrected for the effect of study (Table 2;  $-0.055$  g/g). However, there may be a  
312 biased prediction for ruminants fed close to maintenance requirements (ID#8; black  
313 circles in Figure 4).

314           Finally, when individual data for NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  were independently  
315 adjusted by the random effects of the study, period (within-study) and diet (within-  
316 period and study), their residuals, mainly representing the between-animal variation,  
317 were still negatively correlated with each other ( $P < 0.001$ ) though with a poor fit ( $r^2 =$   
318  $0.12$ ; Figure 4).

319

320 *N partitioning and N isotopic fractionation*

321 Relationships between  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and variables related to N use at the  
322 whole body, rumen, digestive and metabolic levels are presented in Table 3. Overall,  
323  $\Delta^{15}\text{N}_{\text{animal-diet}}$  showed a high degree of correlation with most variables, with  
324 coefficients of determination ( $r^2$ ) ranging from 0.30 (EMPS\_E) to 0.83 (NUE). The  
325  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was negatively correlated to variables reflecting the efficiency of N  
326 utilization at the different levels (EMPU\_prod, EMPU\_tot, DENU, EMPS\_E and  
327 EMPS\_N) while positively correlated to variables reflecting protein degradation in the  
328 rumen (RDP and RPB). Under the experimental conditions of the studies used in this  
329 meta-analysis and according to dietary characterization by the INRA feeding system,  
330 the measured whole-body NUE showed a higher correlation with the efficiency of  
331 metabolic N use ( $r = 0.87$  and  $0.80$  for EMPU\_prod and EMPU\_tot, respectively)  
332 compared with variables reflecting the efficiency of N use in the rumen ( $r = 0.53$  and  
333  $0.43$  for EMPS\_E and EMPS\_N, respectively) or digestive ( $r = 0.61$  for DENU) levels.

334 PLS regression models were developed independently to predict variation in  
335  $\Delta^{15}\text{N}_{\text{animal-diet}}$  or NUE as a function of variables related to N utilization (Figure 5). In  
336 both cases, the best PLS regression models contained three components. These  
337 components explained 96% and 87.0% of the observed  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation (R2Y)  
338 and could predict 93% and 83.0% of variation in new values (predictive ability of the  
339 model; Q2), respectively. The error of prediction of the model was relatively low  
340 (MSEP = 0.017 and 0.44 for NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , respectively) compared to the  
341 range of observed values (Supplementary Table S1). Metabolic N use efficiencies  
342 (EMPU\_tot and EMPU\_prod) were the most important variables in both predictive  
343 models as evidenced by their VIP values (from 1.20 to 1.41 for ENU and from 1.12 to  
344 1.25 for ENU and  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , respectively). In both cases, the VIP scores for the  
345 other variables were lower than the unity for all components. However, when the PLS

346 model for  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was constructed using only these two variables the resulting  
347 predictive performances were much lower (data not shown), highlighting the  
348 importance of all variables for explaining variation in N isotopic fractionation.

349

## 350 **Discussion**

351 High between-animal variability in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  has been reported for  
352 ruminants fed the same diet and reared under the same conditions (Hartman, 2011;  
353 Sponheimer *et al.* 2003). Within our database, we noted that variation in  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
354 for animals reared under identical conditions (same species, study, diet and period)  
355 was as large as 1.7‰ (data not shown), which is half of the accepted trophic shift  
356 value (3.4‰; Minagawa and Wada, 1984), that is the threshold which ecologists use  
357 to distinguish trophic levels. Between-individual variation in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  could stem  
358 from individual differences in protein balance (Sick *et al.*, 1997; Fuller *et al.*, 2004). In  
359 this sense, one of the first authors to evoke the potential link between NUE and  
360  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was Vanderklift and Ponsar (2003). Since then different studies  
361 proposed or confirmed a strong relationship between the N isotopic fractionation and  
362 NUE in fish (Gaye-Siessegger *et al.*, 2004), birds (Sears *et al.*, 2009), pigs (Warinner  
363 and Tuross, 2010) and ruminants (Cheng *et al.*, 2014; Cantalapiedra-Hijar *et al.*,  
364 2015). In the present study, we explored by meta-analysis the relationship between  
365  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE in domestic ruminants through different statistical approaches  
366 and found on average a significant and negative correlation between them, even at  
367 the level of between-animal variation (Table 2 and Figures 3 and 4).

368

369 *Data exclusion from the database*

370 Three dietary treatments belonging to the same study (Cheng *et al.*, 2011)  
371 were first identified as outliers and excluded from our database based on biological  
372 reasons. They had a particular chemical composition (forages with high ammonia-N  
373 content [almost 15% of total N, on average] two fold higher compared to the other 6  
374 experimental diets from the same study) and were associated with high standardized  
375 residuals when exploring the overall and within-study relationship between NUE and  
376  $\Delta^{15}\text{N}_{\text{animal-diet}}$ . One possible explanation for the very low  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values observed  
377 for these three treatments (ranging from 0.81 to 2.06) compared to the rest of diets  
378 (ranging from 2.33 to 3.63) could be differences in N isotopic fractionation by rumen  
379 bacteria depending on the nature of the N source (ammonia vs amino acids; Wattiaux  
380 and Reed, 1997). When data from these three diets were removed a negative rather  
381 than positive (Cheng *et al.* 2011) relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was  
382 found (ID#7 in Figure 2) in accordance with the average trend of other studies in our  
383 database (Cheng *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015). Likewise, a positive  
384 rather than negative relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  can be calculated  
385 from the N balance and isotopic data reported by Sutoh *et al.* (1993) in sheep  
386 receiving diets based on alfalfa hay supplemented or not with sucrose. A different  
387 pattern of N isotopic fractionation at the rumen level when animals are fed diets rich  
388 in rumen degradable protein, as it is the case with alfalfa hay, cannot be excluded  
389 and could agree with the unexpected positive relationship found for three high  
390 ammonia diets removed from our database as discussed above. In the present meta-  
391 analysis, rumen protein balance and rumen degradable protein, both of them  
392 associated with rumen ammonia-N concentration, were moderately and positively  
393 correlated (0.63-0.64) to  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , but they were not, however, the most important  
394 variables explaining  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation in the PLS analysis (Figure 5). Further

395 study is needed to better understand the role of rumen metabolism in N isotope  
396 fractionation when there is a large excess of rumen degradable protein.

397

### 398 *Identifying main sources of variation*

399 The relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  might not be unique across  
400 experimental conditions: the confidence intervals for the estimated slope did not  
401 always overlap across studies and diets. Nevertheless, it is not possible to accurately  
402 predict this relationship for individual diets since the low number of replications led to  
403 inaccurate estimates; that is large confidence intervals as shown in Figure 2. Indeed,  
404 despite a negative response of NUE to variation in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  for most diets, few  
405 significant coefficients were found (9 out of 38). The use of a mixed-effect regression  
406 analysis in our meta-analysis allowed us to estimate an average trend between NUE  
407 and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  without the need to accept that the relationship might be different for  
408 each experimental condition. The need to account for the random effects of known  
409 experimental conditions was thus graphically confirmed and agreed with the previous  
410 intra-class correlation analysis (Table 1) showing the effect of study as a very high  
411 grouping-factor.

412 Despite some results suggesting lower  $\delta^{15}\text{N}$  values in milk vs plasma samples  
413 within the same individual (Jenkins *et al.*, 2001), no effect of sample type was noted  
414 in the overall relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in the present study  
415 (Supplementary figure S1). Finally, because inter-species variation may exist in  
416 rumen and metabolic N utilization, the relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
417 might differ across ruminant species (cattle, goat and sheep). Unfortunately, there  
418 were too few data available in the literature to address this question.

419

420 *Mixed-effect analysis based on treatment means*

421 On average, a global and significant negative relationship between NUE and  
422  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was found under the main influence of dietary treatments (Eq.3 in Table  
423 2; slope = -0.055). The strong impact of diet quality, mainly the protein content and  
424 the nature of the protein, on  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation is well documented in the literature  
425 (Poupin *et al.*, 2011; Vanderklift and Ponsar, 2003). Likewise, dietary protein content  
426 (Huhtanen and Hristov, 2009) and quality (rumen degradable vs undegradable  
427 protein; Hristov *et al.*, 2004) are known to be two of the main determinants of NUE in  
428 ruminants. The slope found with this approach suggests that on average an increase  
429 in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  of 1‰ observed between two dietary treatments is associated with a  
430 0.055 g/g decrease in NUE. This negative slope is compatible with a difference in  
431  $\Delta^{15}\text{N}_{\text{animal-diet}}$  of around 1.5‰ reported by Sponheimer *et al.* (2003a) in cattle fed two  
432 contrasting diets and leading to an estimated difference in NUE (calculated from N  
433 balance) of 0.056 g/g in llamas subsequently fed the same diets (Sponheimer *et al.*,  
434 2003b).

435

436 *Mixed-effect analysis based on individual values*

437 On average, we found a significant and negative relationship between  
438 individual values of NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$ . The discrepancy between the slope  
439 obtained when using individual (-0.035 g/g) rather than treatment mean (-0.055 g/g)  
440 values could stem from the smaller influence, but still significant, of between-animal  
441 variation in the relationship between NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  compared to other  
442 experimental conditions such as physiological animal status or dietary treatments. An  
443 alternative explanation could be that physiological mechanisms explaining between-

444 animal variation in NUE are not exactly the same as those related to between-diet  
445 variation in NUE.

446 Indeed the variance estimates calculated from a simple intercept model  
447 showed the effect of the study as a strong grouping variable explaining more than  
448 half of total variance in NUE and in agreement with previous studies (Kohn *et al.*,  
449 2005; Huhtanen *et al.*, 2015). The high diversity of experimental conditions included  
450 in our database in terms of animal species, physiological status and experimental  
451 diets explained the large contribution of study to NUE variation. However, within a  
452 given study and experimental period it is noted that the variance unexplained by the  
453 diet, and corresponding mainly to between-animal variation, was still very high (35%  
454 and 55% of the NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation, respectively). Thus, by applying a  
455 mixed-effect model to our individual data to take into account the random effects of  
456 the study, diet and period we demonstrated that on average  $\Delta^{15}\text{N}_{\text{animal-diet}}$  is  
457 negatively related to between-animal variation in NUE, which otherwise would have  
458 been only evident for 9 out of 38 diets (Figure 2).

459

#### 460 *Limits of the prediction model*

461 A generalizable equation might result in a biased estimation of NUE,  
462 especially when animals are in a negative or close to zero N balance as it is the case  
463 for data from the only study carried out on adult ruminants fed close to maintenance  
464 energy requirements (ID#8 represented as black circles in Figure 3). Data from that  
465 study seem to be responsible for a slightly better fitting when a quadratic term was  
466 included in the unadjusted between-study relationship of NUE vs  $\Delta^{15}\text{N}_{\text{animal-diet}}$  (Figure  
467 1a; data not shown). The sharper slope in the relationship between NUE and  
468  $\Delta^{15}\text{N}_{\text{animal-diet}}$  found in the study ID#8 (Figure 2) supports the concept of a higher  $^{15}\text{N}$

469 enrichment over the diet when the main protein source for the individual is its own  
470 stores (Martinez del Rio *et al.*, 2009) as found for example when birds lose weight  
471 during egg-laying and incubation (Hobson *et al.*, 1993), when feeding levels were  
472 below maintenance requirements in fish (Gaye-Siessegger *et al.*, 2007) or when  
473 pregnant women lose weight during nutritional stress (Fuller *et al.*, 2014). However,  
474 we contend that this does not preclude the possibility of using  $\Delta^{15}\text{N}_{\text{animal-diet}}$  to rank  
475 such animals for NUE. Future studies should investigate effects of body N reserve  
476 mobilization on relationships with  $\Delta^{15}\text{N}_{\text{animal-diet}}$ .

477

#### 478 *Mixed-effect regression based on residuals*

479 A difference in  $\Delta^{15}\text{N}_{\text{animal-diet}}$  values of 1‰ for animals fed the same diet at the  
480 same time (and site) was associated on average with a significant difference of 0.024  
481 g/g in NUE, which represent around 10% of the mean value of NUE obtained in our  
482 database (Supplementary Table S1). This result highlights the ability to use this new  
483 isotopic biomarker to rank animals reared under similar conditions for NUE, although  
484 with a greater uncertainty than for diets, and it agrees with results obtained by Gaye-  
485 Siessegger *et al.* (2004) in 32 fish showing large between-animal variation in NUE  
486 (from around -0.05 to around 0.40 g/g). Our finding also agrees with the significant  
487 and negative relationship found by Wheadon *et al.* (2014) when  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was  
488 used as a predictor of between-animal variation in feed conversion efficiency  
489 (average daily gain/dry matter intake) in 84 growing beef heifers fed the same diet at  
490 the same time (slope = -0.014 g/g FCE). The relatively sharper slope found in the  
491 present study compared to that found by Wheadon *et al.* (2014) could be explained  
492 by the fact that lipids contribute to body weight gain, and therefore to feed conversion  
493 efficiency, but not to NUE.

494

495 *Link between N partitioning and N isotopic fractionation*

496 In the present study, the two variables related to the N use efficiency at the  
497 metabolic level (EMPU\_tot and EMPU\_prod) were the most important parameters,  
498 based on their VIP values, explaining both NUE and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation. These  
499 results agree with two studies conducted in rats (Sick *et al.*, 1997; Poupin *et al.*, 2014)  
500 and showing that the  $\Delta^{15}\text{N}_{\text{animal-diet}}$  correlated well with the balance between protein  
501 synthesis vs catabolism in the liver, an indicator of metabolic N efficiency use.  
502 Although there is clear evidence of a N isotopic fractionation carried out by rumen  
503 bacteria (Sutoh *et al.*, 1993; Wattiaux and Reed, 1995) our findings suggests that on  
504 average they would contribute to a lesser extent to  $\Delta^{15}\text{N}_{\text{animal-diet}}$  variation than  
505 metabolic processes. This result agrees with the concept of a higher contribution of  
506 animal metabolism to between-animal variation in feed efficiency compared to other  
507 determinants such as digestion, heat increment of feeding or feeding pattern  
508 (Richardson and Herd, 2004). This is likely one of the main reason why milk urea-N  
509 concentration may be unable to capture properly between-animal variation in NUE in  
510 ruminants (Vallimont *et al.*, 2011; Huhtanen *et al.* 2015), since their values have been  
511 mostly correlated to rumen N losses (Nouisiainen *et al.*, 2004, Hof *et al.*, 1997) rather  
512 than to the efficiency of metabolic N use (Hof *et al.*, 1997).

513 Our results may suggest that the ability of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  to describe the  
514 between-animal variation in NUE can be due to the strong link between the N isotopic  
515 fractionation and metabolic use of N. Finally, one strength of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  is the  
516 virtually lack of diurnal variation and its stability irrespective of the feeding time given  
517 its slow turnover rate. This can be advantageous when seeking to predict NUE in  
518 animals adapted to their diets over long periods, but showing different daily feeding

519 patterns. In contrast, the period of time between the introduction of a new diet and  
520 the blood/milk sampling is an issue when predicting NUE through  $\Delta^{15}\text{N}_{\text{animal-diet}}$   
521 values, as revealed in our meta-analysis (the effect of period as a grouping-factor),  
522 since it takes a long time to reach a new isotopic equilibrium (proposed lag of 45  
523 days for plasma proteins in ruminants, Cantalapiedra-Hijar *et al.*, 2015). Future  
524 research should evaluate the complementarity between MUN and  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and  
525 explore the natural abundance of  $^{15}\text{N}$  in specific N compounds (such as individual  
526 amino acids) to better predict NUE.

527

## 528 **Conclusions**

529 We showed through a meta-analysis approach that the natural  $^{15}\text{N}$  enrichment of  
530 ruminant protein over the consumed diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) is on average significantly  
531 and negatively correlated to N use efficiency in domestic ruminants reared under  
532 different conditions. This correlation was significant even at the between-animal level,  
533 highlighting the ability of this isotopic biomarker to rank individuals from a  
534 homogenous group (same animal species, physiological status and fed the same diet  
535 at the same time) in terms of N use efficiency. The most important variables related  
536 to N utilization explaining the link between  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and NUE under the studied  
537 conditions were those related to N metabolism, with rumen fermentation and  
538 digestion contributing to a lesser extent.

539

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543

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**Table 1.** Variance-component estimates of N use efficiency (NUE) and N isotopic fractionation ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) in ruminants when random effects of study (ID), period within-study (ID/P) and diet within-period and study (ID/P/D) were considered.

	Average value ( $\beta_0$ )	Estimate ( $\sigma$ )	95% CI ( $\sigma$ )	ICC <sup>1</sup> (%)
NUE, g/g (n= 217)	0.235±0.029			
ID		0.091	0.057-0.145	75.5
ID/P		0.030	0.018-0.051	33.6
ID/P/D		0.034	0.027-0.043	64.9
Residual <sup>2</sup>		0.025	0.022-0.027	
$\Delta^{15}\text{N}_{\text{animal-diet}}$ , ‰ (n = 282)	3.23±0.42			
ID		1.37	0.87-2.16	84.9
ID/P		0.33	0.21-0.53	32.6
ID/P/D		0.32	0.24-0.42	45.5
Residual		0.35	0.31-0.38	

<sup>1</sup>Intra-class correlation coefficient: total variance explained by the tested random variables. For nested random variables as ID/P/D for instance it refers to the variance explained by the dietary treatment unexplained by the experimental period and study.

<sup>2</sup>Total variance ( $\sigma$ ) unexplained by the random effect of study, period and diet (within-group variability) and including mainly the between-animal variability and experimental error.

Table 2. Mixed-effect regression models of N use efficiency in ruminants (Y) on the N isotopic fractionation (X) using either treatment means or individual values<sup>†</sup>

	Intercept	Slope	RSE <sup>1</sup>	AIC <sup>1</sup>	BIC <sup>1</sup>	Variance component estimates ( $\sigma$ )						
						ID		P		D		
						Intercept	Slope	Intercept	Slope	Intercept	Slope	
Treatment means (n = 38)												
Random-effects: (Eq.3) ID <sup>2</sup>	0.415*±0.057	-0.055*±0.007	0.029	-139	-132	1.9e-6	0.008					
Individual values (n = 217)												
Random-effects: (Eq.4) ID <sup>2</sup>	0.420*±0.057	-0.050*±0.014	0.033	-1021	-1002	0.182	0.044					
(Eq.5) ID/P <sup>2</sup>	0.380*±0.015	-0.042*±0.005	0.032	-1064	-1038	0.029	0.010	0.006	0.007			
(Eq.6) ID/P/D <sup>2§</sup>	0.358*±0.014	-0.035*±0.005	0.022	-1117 <sup>§</sup>	-1084 <sup>§</sup>	0.024	0.010	1.9e-6	0.005	0.020	0.007	

<sup>†</sup> All models were tested with random effects on the intercept, slope or both. Best models were obtained when random effects on intercept and slope were included

<sup>1</sup> RSE = residual standard error; AIC = Akaike information criterion; BIC = Bayesian information criterion

\*: *P*-value < 0.001

<sup>2</sup> ID = random effect of the experimental study; ID/P = random effect of period within the experimental study; ID/P/D = random effect of diet within period and experimental study

<sup>§</sup> Best random structure model based on AIC/BIC criteria and the log-likelihood ratio test (*P* < 0.05). Comparisons were conducted with the maximum likelihood method and when using individual values.

**Table 3.** Pearson correlation coefficients between nitrogen use efficiency (NUE), nitrogen isotopic fractionation ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) and parameters related to N utilization in ruminants.

	NUE <sup>1</sup>	EMPU_prod <sup>1</sup>	EMPU_tot <sup>1</sup>	RDP <sup>1</sup>	RPB <sup>1</sup>	EMPS_E <sup>1</sup>	EMPS_N <sup>1</sup>	DENU <sup>1</sup>	
$\Delta^{15}\text{N}_{\text{animal-diet}}$ , ‰	-0.91	-0.76	-0.73	0.64	0.63	-0.55	-0.57	-0.66	
NUE <sup>1</sup> , g/g		0.87	0.80	-0.55	-0.57	0.43	0.53	0.61	
EMPU_prod <sup>1</sup> , g/g			0.89	-0.19	-0.19	0.10	0.13	0.19	
EMPU_tot <sup>1</sup> , g/g				-0.36	-0.34	0.16	0.25	0.21	
RDP <sup>1</sup> , g/kg DM					0.99	-0.66	-0.91	-0.89	1
RPB <sup>1</sup> , g/kg DM						-0.63	-0.94	-0.93	
EMPS_E <sup>1</sup> , g/kg fOM							0.63	0.68	R
EMPS_N <sup>1</sup> , g/kg RDP								0.95	D
									P

= 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

**Figure 1.** Relationship between N use efficiency and N isotopic fractionation ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) in ruminants using individual values (n = 282). a) Simple linear regression analysis (*overall relationship*:  $\text{NUE} = 0.429 - 0.058 \times \Delta^{15}\text{N}_{\text{animal-diet}}$ ) where open triangle = dairy cows; open circles = dairy goats; closed triangles = non-lactating sheep; closed circles = growing beef cattle, b) Simple linear regression for each study (n = 11; *within-study regression*), c) Simple linear regression analysis for each diet (n = 38; *within-diet regression*). Coefficients for individual regressions within-study and within-diet are presented in Figure 2.

**Figure 2.** Confidence intervals (95%) for the intercepts and slopes obtained from the regression of N use efficiency (Y) in ruminants on N isotopic fractionation (X). a): Within-study regression (ID#); b): Within-diet regression (ID#\_T). When non-significant estimates were found, the letters *NS* appears beside the corresponding confidence interval. All slopes are numerically negative within-study (a) and 31 out of 38 within-diet (b). However, large confidence intervals are obtained because of the relative low number of observations within-study (n= 15 to 34) and within-diet (n= 3 to 16) leading to non-significant responses in 4 studies out of 11 and in 29 dietary treatments out of 38. The average slope was -0.053 and -0.046 within-study and within-diet, respectively, compared to a slope of -0.058 between studies (Fig 1).

**Figure 3.** Overall relationship between N use efficiency in ruminants (N retention or milk N secretion/N intake) and N isotopic fractionation ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) when taking into account the random effects of the study, diet (within-study) and period (within-diet) on the intercept and slope. The resulting equation is:  $\text{NUE} = 0.358 (\pm 0.014) - 0.035 (\pm 0.0050) \times \Delta^{15}\text{N}_{\text{animal-diet}}$  (RSE = 0.022). Black circles identify individual data from the only study (ID#8) using non-productive animals. Dashed lines depict 95% confidence intervals of the regression equation.

**Figure 4.** Simple linear regression between residuals of N use efficiency in ruminants (NUE) and N isotopic fractionation ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) obtained when both variables were independently adjusted for the random effects of the study, period (within-study) and diet (within-period and study). Equation:  $\text{NUE} = -0.024 (\pm 0.0039) \times \Delta^{15}\text{N}_{\text{animal-diet}}$  ( $P < 0.001$ ;  $R^2 = 0.12$ , RSE = 0.019).

**Figure 5.** Partial Least Squares (PLS) regression analysis of either a) NUE or b)  $\Delta^{15}\text{N}_{\text{animal-diet}}$  on descriptors of ruminant N partitioning (Efficiency of metabolizable protein use for production [EMPU\_prod]; Efficiency of metabolizable protein use for total net protein synthesis [EMPU\_tot]; Rumen degradable protein [RDP; Rumen protein balance [RPB]; Efficiency of microbial protein synthesis according to the available energy [EMPS\_E]; Efficiency of microbial protein synthesis according to rumen degradable protein [EMPS\_N]; Digestive efficiency of N use [DENU]). Best PLS regression model kept three components (t) in both cases with Q2 values ranging from 0.83 ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) to 0.92 (NUE). The most important variables according to VIP (variable importance in projection) were in both cases EMPU\_prod (1.17 to 1.41) and EMPU\_tot (1.12 to 1.24) and are highlighted in bold type in both graphics.

