

# Natural $^{15}\text{N}$ abundances in plasma and urea-N concentration in milk as biomarkers of urinary N excretion in dairy cows: a meta-analysis

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## Abstract

Knowledge on the flux of nitrogen (N) excreted through urines is essential in animal nutrition since it may provide information on animal N metabolism and the extent of environmental N pollution. Both the  $^{15}\text{N}$  natural enrichment of animal proteins over the diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) and the milk urea N (MUN) concentration have been previously linked to N partitioning. The objective of this meta-analysis was to test the effect of combination of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in plasma and MUN for predicting the urinary N excretion (UNE) in dairy cows. Individual data on  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in plasma, MUN, and UNE of 177 cow/period observations were evaluated by meta-analysis. Data were analyzed by mixed-model regression analysis. When model was fitted with either  $\Delta^{15}\text{N}_{\text{animal-diet}}$  or MUN, both biomarkers were capable to independently predict UNE. However, when  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and MUN were combined in one model, both biomarkers remained significant and the error of the model decreased. In conclusion, both  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and MUN can predict UNE and their combination can strengthen the prediction capacity of the model.

**Keywords:** biomarkers, N metabolism, N pollution, cattle

## Introduction

In most feeding conditions, urinary N excretion (UNE) is a major source of N loss in ruminants. Given the difficulty to measure UNE, researchers are exploring biomarkers to predict it. A promising biomarker of N utilization based on the natural  $^{15}\text{N}$  enrichment of animal proteins over the diet ( $\Delta^{15}\text{N}_{\text{animal-diet}}$ ) was explored. The relationship was likely due to both rumen bacteria and liver enzymes discriminate against  $^{15}\text{N}$  during N assimilation and catabolism (Cantalapiedra-Hijar et al., 2019 [abstract #XX]; Sick et al., 1997). A traditional biomarker for predicting UNE is the milk urea N (MUN) concentration, which seems to be more adapted to sign the digestive rather than the metabolic component of UNE variations (Hof et al., 1997). The objective of this meta-analysis was to test the effect of combination of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in plasma and MUN for predicting UNE in dairy cows.

## Material and methods

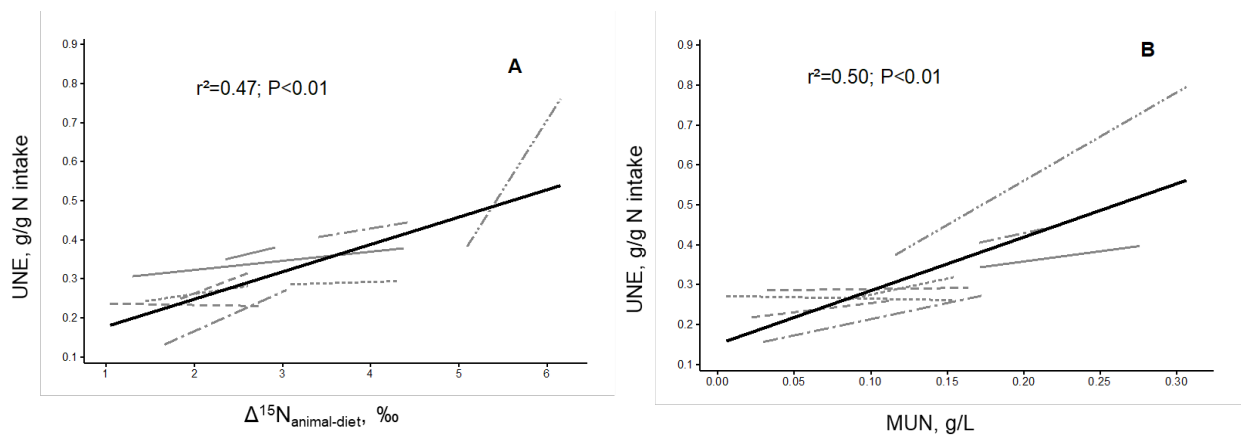
Individual data on  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in plasma proteins, MUN, and UNE (g/g N intake measured from total collection) of 177 cow/period observations from 86 dairy cows fed 34 different diets and from 9 different experiments were evaluated by meta-analysis. Eight experiments were on lactating animal and one with heifers in which the values of blood urea N was used instead of MUN. The average values for  $\Delta^{15}\text{N}_{\text{animal-diet}}$ , MUN, and UNE were  $3.3 \pm 1.06\%$ ,  $0.1 \pm 0.06$  g/L, and  $0.33 \pm 0.115$  g/g N intake, respectively. Data were analyzed by mixed-model regression analysis and the random structure of the model was evaluated by testing different nested effects (experiment, period, and diet) on both the intercept and slope and keeping the one with the lowest AIC and log likelihood ratio. Values for variance inflation factor were checked when combining the two biomarkers to avoid multi-collinearity problems.

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## Results and discussion

Overall, the relationship between UNE and either  $\Delta^{15}\text{N}_{\text{animal-diet}}$  or MUN was significant and positive correlations in both cases with a similar model fitting (Figure 1). The result of mixed model analysis showed that when  $\Delta^{15}\text{N}_{\text{animal-diet}}$  was the only predictor of UNE, the best-fitted model was  $\text{UNE (g/g N intake)} = 0.18 (\pm 0.025) + 0.05 (\pm 0.011) \Delta^{15}\text{N}_{\text{animal-diet}} (\text{‰})$  (SD of residual = 0.046;  $P < 0.01$ ). When MUN was the only predictor of UNE the best-fitted model was  $\text{UNE (g/g N intake)} = 0.29 (\pm 0.040) + 0.31 (\pm 0.142) \text{MUN (g/L)}$  (SD of residual = 0.048;  $P < 0.01$ ). Finally, when both biomarkers were used as predictors, both  $\Delta^{15}\text{N}_{\text{animal-diet}}$  ( $P < 0.01$ ) and MUN ( $P = 0.01$ ) significantly contributed in the prediction equation and the error of the model marginally decreased:  $\text{UNE (g/g N intake)} = 0.15 (\pm 0.027) + 0.04 (\pm 0.013) \Delta^{15}\text{N}_{\text{animal-diet}} (\text{‰}) + 0.34 (\pm 0.131) \text{MUN (g/L)}$  (SD of residual = 0.045;  $P < 0.01$ ). The result of our study is in line with our hypothesis on the improvement of UNE prediction by combining  $\Delta^{15}\text{N}_{\text{animal-diet}}$  and MUN. A recent meta-analysis highlighted the potential of  $\Delta^{15}\text{N}_{\text{animal-diet}}$  to reflect metabolic rather than rumen variability in N utilization (Cantalapiedra-Hijar et al., 2018), while MUN is a well-known biomarker for detecting dietary effect on N utilization and signing the digestive component of UNE (Hof et al., 1997). The combination of both biomarkers would have a synergy effect on the prediction of UNE, because of their complementarity in signing different mechanisms underlying UNE and N utilization. In conclusion,  $\Delta^{15}\text{N}_{\text{animal-diet}}$  in plasma and MUN may be combined to improve the prediction of UNE in dairy cattle.



**Figure 1.** Relationships between urinary N excretion (UNE; g/g N intake) and either A)  $\Delta^{15}\text{N}_{\text{animal-diet}}$  (‰) or B) milk urea-N (MUN; g/L). Each individual line represents the within-study regression whereas the thick black line represents the overall between-study regression.

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