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Identifying cattle with superior growth feed efficiency through their natural ^{15}N abundance and plasma urea concentration: A meta-analysis

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Abstract

The objective of this study was to test two candidate biomarkers of feed efficiency in growing cattle. A database was built using performance data from 13 trials conducted with growing heifers, steers and young bulls and testing 34 dietary treatments. Different breeds were used with Charolais (37%), Simmental (15%), and cross-bred (40%) cattle being the most numerous. The database included 759 individual records for animal performance and laboratory data for N isotopic discrimination measured in plasma or muscle ($\Delta^{15}N_{animal\text{-}diet}$; n = 749) and plasma urea concentration (n = 659). Feed conversion efficiency (FCE) and residual feed intake (RFI) criteria were calculated for a duration ranging between 56 and 259 d, depending on the trial. For FCE prediction, mixed models included the random effects of study, treatment within-study and pen within-study (i.e. contemporary group; CG) allowing these effects to be progressively excluded from the relationship. For RFI prediction, simple linear regressions were tested with the CG effect removed from biomarker values before analysis. Better models were obtained with $\Delta^{15}N_{animal\text{-}diet}$ compared to plasma urea concentration, irrespective of using mean or individual values and regardless of the feed efficiency criterion. Prediction error (0.027 kg/kg) from mixed-effect models using mean FCE and $\Delta^{15}N_{animal\text{-}diet}$ values would allow discrimination of 2 dietary treatments or production conditions in terms of FCE if they differ by more than 0.10 kg/kg. The $\Delta^{15}N_{animal\text{-}diet}$ values showed a negative and significant ($P<0.001$) relationship with FCE at the individual level and results highlighted that it is possible to significantly discriminate two animals randomly selected from the same CG if they differ by at least 0.06 kg/kg FCE. In addition, the top 20% highest and lowest animals within-CG in terms of RFI and FCE (extreme animals) showed significant ($P<0.001$) differences in $\Delta^{15}N_{animal\text{-}diet}$ values, while only extreme FCE animals could be discriminated when using plasma urea concentrations ($P=0.002$). No gain in feed efficiency prediction was observed when combining candidate biomarkers. However, when average daily gain data was combined with $\Delta^{15}N_{animal\text{-}diet}$, the prediction of FCE at the individual level was strengthened compared to using only one of them, in which case average daily gain was the best single predictor. Our findings confirm that $\Delta^{15}N_{animal\text{-}diet}$ may be useful to form groups of animals for precision feeding when feed intake and body weight gain are not available. Further studies are warranted, however, to evaluate the usefulness of this promising biomarker for genetic selection.

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Contents

Introduction

Identifying individuals within a herd with greater ability to convert feed into body weight (i.e. superior growth feed efficiency) may have positive consequences on the profitability and sustainability of the beef cattle sector (Cruz et al., 2010; Basarab et al., 2013). Benefits could arise from both genetic selection and precision feeding. Efficient cattle consume less feed to reach the same market body weight (Berry and Crowley, 2013). Because animal feed efficiency is moderately heritable, identification and selection of superior individuals for breeding can improve this trait in future generations (Taussat et al., 2019). Furthermore, substantial gains could be also achieved if nutrition is adapted to individual requirements (i.e. precision feeding). In this regard, feed resources and expensive nutrients could be strategically allocated to certain animals with specific requirements within a herd. Indeed, precision feed restriction (Fischer et al., 2020) or targeted changes in the forage to concentrate ratio (Meir et al., 2021) applied to cattle eating beyond their theoretical requirements were recently shown to be promising strategies to improve feed efficiency. These precision feeding strategies are logically dependent on an accurate prior ranking of animals in terms of feed efficiency.

For both genetic and precision feeding purposes there is a need to develop predictors that may easily rank animals within a herd in terms of feed efficiency where individual feed intake and body weight recording, the basic traits for calculating feed efficiency, are not available on farm. Although current ruminant feeding systems may roughly predict the average feed efficiency for a group of animals fed the same diet (INRA, 2018), there are not yet validated predictors or biomarkers available on farm for ranking individual animals in terms of feed efficiency around the group average. This may be due to the difficulty of identifying common biological mechanisms underlying between-animal variation in growth feed efficiency across different type of diets (Jorge-Smeding et al., 2021), age and breeds (Karisa et al., 2014; Carmelo et al., 2020). Among potential mechanisms, some recent research pointed to N metabolism as a key process explaining between-animal variation in feed efficiency (Cantalapiedra-Hijar et al., 2020b). Growth feed efficiency and N use efficiency (**NUE**; i.e. nitrogen retention/N intake) are biologically linked in growing ruminants considering that body weight gain and N retention are highly correlated traits (Geay, 1984). Moreover, because feed sorting is negligible with most fattening diets, total feed intake and N intake

are expected also to be highly correlated. Indeed, recent studies highlighted that plasma biomarkers of N partitioning and NUE may also serve to track between-animal variability in feed efficiency in fattening young bulls fed contrasting diets (Cantalapiedra-Hijar et al., 2020b; Jorge-Smeding et al., 2021; Guarnido-Lopez et al., 2021). This means that biomarkers predicting differences in NUE could equally work for discriminating individuals in terms of feed efficiency.

In this regard, a new biomarker of NUE for ruminants, based on the N isotopic discrimination naturally occurring between the animal and its diet (**$\Delta^{15}N_{animal\text{-}diet}$**), has been confirmed by meta-analysis (Cantalapiedra-Hijar et al., 2018a) with the potential to identify differences between-animals in feed efficiency (Guarnido-Lopez et al., 2021). Briefly, some enzymatic reactions occurring at the rumen (ammonia uptake and amino acid [**AA**] catabolism by rumen bacteria; Wattiaux and Reed, 1995) and hepatic (AA catabolism; Cantalapiedra-Hijar et al., 2015) levels have a higher affinity for ^{14}N vs ^{15}N compounds, leading to urine and animal proteins that are naturally depleted and enriched in ^{15}N, respectively. The greater the ratio of AA catabolism to urea-N uptake by rumen bacteria and the hepatic AA catabolism to protein synthesis ratio (i.e. lower NUE) the greater the natural ^{15}N enrichment of animal proteins over the consumed diet (i.e. greater $\Delta^{15}N_{animal\text{-}diet}$) (for details see Cantalapiedra-Hijar et al., 2016). This isotopic biomarker can reveal changes in N partitioning in many different animal species (Gaye-Siessegger et al., 2004; Sears et al., 2009), as well as in humans (Fuller et al., 2004). In the last decade, several ruminant studies applied this isotopic biomarker to rank diets and individuals in terms of feed conversion efficiency with most correlations being high to moderate (Wheadon et al., 2014; Cantalapiedra-Hijar et al., 2015; Meale et al., 2017). However, the few studies attempting to correlate $\Delta^{15}N_{animal\text{-}diet}$ to residual feed intake (**RFI**) failed to prove significant relationships (Wheadon et al., 2014; Meale et al., 2017). Although we have recently validated the ^{15}N natural abundance in plasma as a biomarker of feed efficiency variation in Charolais young bulls from the same contemporary group (Guarnido-Lopez et al., 2021), the generalization of the relationship between $\Delta^{15}N_{animal\text{-}diet}$ and feed efficiency across different experimental conditions (dietary treatments and breeds) has not yet been explored.

Therefore, this study used meta-analyses to assess the extent to which $\Delta^{15}N_{animal\text{-}diet}$ can be recommended as a generalizable predictor of growth feed efficiency variation in cattle, across different dietary and production conditions, as well as at the level of between-animal variation, and according to feed conversion efficiency and residual feed intake criteria. Given that plasma or blood urea has been found and proposed as a biomarker of NUE (Kohn et al., 2005) and feed efficiency in beef cattle (Richardson et al., 2004), we compared the ability of both biomarkers ($\Delta^{15}N_{animal\text{-}diet}$ and plasma urea) to discriminate dietary treatments and individuals in terms of feed efficiency across a variety of feeding and fattening production systems in Europe.

Material and methods

Experimental data

Individual observations for animal performance data and $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea analysis were recovered from published (ID#1to ID#7) or unpublished (ID#8 to ID#13) feed efficiency studies in growing-fattening cattle (Table 1) conducted across 4 EU countries (France, United Kingdom, Ireland and Switzerland).

Overall, the database comprised of 13 experimental studies, 34 dietary treatments and individual animal values ($n_{\_indiv}$ = 759) for feed conversion efficiency (**FCE**) and RFI, $\Delta^{15}N_{animal\text{-}diet}$ ($n_{\_plasma}$ = 659 and $n_{\_muscle}$= 90) and plasma urea ($n_{\_indiv}$ = 659) measured in young growing bulls (9 studies), steers (3 studies) and heifers (1 study). Feed efficiency tests lasted from 56 d (steers; ID#5 and ID#13) to 259 d (young bulls; ID#8) with an average of 136±75 d. Blood was always sampled at the end of the feed efficiency test, except for ID#8 where muscle instead of blood was sampled at the slaughterhouse once the feed efficiency test was finished. According to previous results from our team (Nasrollahi et al., 2020) and when sufficient time to reach an isotopic equilibrium, $\Delta^{15}N_{animal\text{-}diet}$ measured from either muscle (#ID8) or plasma proteins have the same potential to predict feed efficiency and thus were treated similarly in this meta-analysis. Different breeds were used, with Charolais being the most numerous pure breed in terms of number of studies ($n_{\_study}$ = 7) or individual observations ($n_{\_indiv}$ = 280), followed by Simmental ($n_{\_indiv}$ = 112).

Table 1. Description of the experimental studies included in the database in terms of type of growing cattle, number of animals, number and type of diets as well as basic descriptive statistics for feed efficiency (FCE; dependent variable) and the isotopic N fractionation ($\Delta^{15}N_{animal-diet}$; independent variable)

					FCE, kg/kg				$\Delta^{15}N_{plasma-diet}$, ‰
	Breed[1]/Type of Animal/Age[2]	#Anim	#Diets	Dietary treatment[3]	Length, d	Mean ± SD	Min	Max	Mean ± SD
Trials for model construction									
ID#1	SIM or SIMx/heifers/	84	1	70%GS+30%CS	84	0.09±0.03	0.03	0.16	4.24±0.34
(Wheadon et al., 2014)	10-13 months								
ID#2	CH/young bulls/	8	4	55%CS+45%CS	184	0.21±0.01	0.19	0.22	3.50±0.38
(Cantalapiedra-Hijar et al., 2015)	11-17 months	9		65%CS +35%CS	184	0.22±0.02	0.19	0.24	3.10±0.25
		9		40%GS+60%CF	184	0.18±0.01	0.16	0.20	3.66±0.38
		8		50%GS+50%CF	184	0.17±0.01	0.14	0.18	3.92±0.18
ID#3	CH/young bulls/	20	2*	70%GS+30%CS	99	0.21±0.02	0.18	0.25	3.76±0.26
(Meale et al., 2017)	10-13 months	34		70%GS+30%CS	99	0.20±0.02	0.16	0.25	3.79±0.31
ID#4	CHx or LU/steers/	40	2	8%Straw+92%CS	56	0.15±0.02	0.10	0.20	2.90±0.27
(Duthie et al., 2017)	13-15 months	40		50%MS+50%CS	56	0.15±0.02	0.10	0.18	2.74±0.38
ID#5	ANGx or LIMx/steers/	20	4	55%MS+45%CS	56	0.15±0.01	0.13	0.17	3.60±0.25
(Meale et al., 2018)	13-15 months	20		55%MS+45%CS+Lip	56	0.15±0.01	0.12	0.18	3.68±0.30
		20		55%MS+45%CS+Nit	56	0.13±0.01	0.11	0.16	4.23±0.35
		20		55%MS+45%CS+LipN	56	0.14±0.01	0.11	0.16	4.39±0.26
ID#6	CH/young bulls/	12	2	65%GS + 35%CS	240	0.16±0.01	0.14	0.18	4.04±0.37
(Nasrollahi et al., 2020)	11-19 months	12		65%CS + 35%CF	240	0.19±0.02	0.15	0.21	3.19±0.41
ID#7	CH/young bulls/	8	4	60%GS + 40%CFLP	220	0.19±0.02	0.15	0.21	2.63±0.15
(Cantalapiedra-Hijar et al., 2020a)	9-17 months	8		60%GS + 40%CFLP[$]	220	0.19±0.02	0.16	0.23	2.46±0.26
		9		60%GS + 40%CFHP	220	0.19±0.02	0.17	0.21	2.37±0.25
		9		60%GS + 40%CFHP[$]	220	0.21±0.02	0.19	0.24	2.36±0.16
ID#8	LIMx, SIMx, MO/young	15	6	70%CS+30%CSBM	259	0.22±0.02	0.19	0.26	2.73±0.29[#]
(unpublished)	bulls/5-13 months	15		60%CS + 40%CRSM+Pea	259	0.20±0.01	0.18	0.22	3.24±0.23[#]
		15		63%CS + 37%CL	259	0.20±0.01	0.17	0.23	3.27±0.21[#]
		15		70%MS + 30%CL	259	0.21±0.01	0.19	0.22	3.19±0.19[#]
		14		63%MS + 37%CPea	259	0.20±0.01	0.18	0.22	3.02±0.24[#]
		14		70%CSSh + 30%CSBM	259	0.21±0.02	0.16	0.25	2.73±0.29[#]
ID#9	ANGx, LIMx, SIMx/young	59	2	70%CS + 30%CPS	85	0.25±0.02	0.19	0.28	2.04±0.18
(unpublished)	bulls/5-8 months	30		80%MS + 20%CPS	85	0.26±0.02	0.20	0.29	1.89±0.28
ID#10	CH/young bulls/	25	2	65%CS+35%CRS	85	0.18±0.02	0.13	0.21	3.17±0.26
(unpublished)	10-13 months	26		65%GS+35%CRF	85	0.16±0.02	0.13	0.20	2.71±0.23
ID#11	CHx, ANGx, SAL/young	33	1	65%GS+35CRF	112	0.15±0.02	0.09	0.19	2.87±0.25
(unpublished)	bulls/12-16 months								
ID#12	CH/young bulls/11-14 months	21	2	55%CS + 45%CRPS	104	0.20±0.02	0.16	0.24	1.97±0.17
(unpublished)		22		55%CS + 45%CRPS[$]	104	0.20±0.02	0.16	0.25	2.08±0.21
ID#13	ANGx, LIMx/steers/14-16	31	2	55%GS+45%CRS	56	0.14±0.02	0.08	0.20	3.25±0.43
(unpublished)	months	33		55%GS+45%CRS[$]	56	0.15±0.02	0.11	0.19	3.21±0.41
TOTAL		**759**	**34**		**136±75**	**0.17±0.05**	**0.03**	**0.29**	**3.13±0.80**

* Two diets because even if diet formulation was similar the experiment was conducted in two different years; [$] Same diet with an experimental additive to be tested; [#] Natural ^{15}N abundances were measured in muscle sampled at the slaughterhouse rather than in plasma

[1]ANG = Angus, CH= Charolais, LIM = Limousin, LU = Luing, MO = Montbeliard, SAL = Salers, SIM = Simmental, ANGx = crossed with Angus, CHx = crossed with Charolais, LIMx = crossed with Limousin, SIMx = crossed with Simmental; [2]Average interval age between the beginning and the end of the feed efficiency test; [3]GS: mostly grass silage; CS: mostly corn silage, MS = mixed (grass, Luzerne, beet pulp or corn) silage, CRS= concentrate rich in starch; CRSF = concentrate rich in starch and fat; CRF = concentrate rich in fiber; CRPS = concentrate rich in protein and starch; Lip = oil supplement; Nit = nitrate supplement; LipNit = Oil and nitrate supplement; CFLP = concentrate rich in fiber and low in protein, CFHP = concentrate rich in fiber and high in protein, CSBM = concentrate including soybean meal, CRSM = concentrate including rapeseed meal, CL = concentrate including lupine, CPea = concentrate including pea, CRSM+Pea = concentrate including both rapeseed meal and pea

Cross-bred represented 40% of total observations and were based mostly on crosses from Angus, Charolais, Simmental and Limousin (Table 1). A large variety of dietary treatments were included in the database, with almost half of the diets being rich in grass silage (≥50%DM). A description of the experimental studies, dietary treatments and animal breeds, used in our database is presented in Table 1.

Feed efficiency calculations

Two different feed efficiency traits were used in this study. Feed conversion efficiency was calculated for each animal within-study as the ratio between average daily gain (**ADG**) and daily dry matter intake (**DMI**), both individually measured for the same period in all trials. Technology for measuring the individual daily DMI varied across studies, with individual Calan gate feeders (American Calan Inc., Northwood, NH) used in study ID#1, Insentec electronic feeders (Insentec BV, Marknesse, The Netherlands) in studies ID#4, ID#5, ID#8, ID#9 and ID#13, electronic dairy gates (Dairy gate, I.F.E.I.,Villeroy, France) in studies ID#2, ID#3, ID#7 and automatic intake recoding systems based on mangers placed on weigh cells (Biocontrol, Rakkestad, Norway) in studies ID#6, ID#10, ID#11, ID#12. For each study, the ADG of each animal was calculated as the slope of the regression of body weight on time, with body weight recording conducted every week, fortnightly or twice per month depending on the study. Residual feed intake (RFI) was calculated from the whole database as the difference between observed and predicted dry matter intake within-contemporary group (**CG**), the latter defined as the group of animals from the same study, fed the same diet and sharing the same pen (n_contemporary group = 58). The number of animals per CG averaged 13 ranging from 5 (ID#2) to 34 (ID#3). For the RFI model, observed DMI was predicted from the CG effect, average daily gain (ADG) and mean metabolic body weight ($mBW^{0.75}$), the latter calculated at the middle of the feed efficiency test. The RFI model was as follows:

$$Y_{ij} = \beta_0 + CG + \beta 1(mBW^{0.75}) + \beta 2\ (ADG) + \varepsilon_{ij}$$

where Y_{ij} is the observed individual daily DMI, β_0 is the intercept, CG corresponds to the contemporary group effect, $\beta 1$ is the regression coefficient for $\mathrm{mBW}^{0.75}$, $\beta 2$ is the regression coefficient for ADG, and ε_{ij} is the residual of the model, defined as RFI.

The predictors of the RFI model (CG, ADG, $mBW^{0.75}$) explained 90% of the observed DMI variation. Forty-two percent of the observed DMI variation within-CG was explained by animal performance (ADG, $mBW^{0.75}$), while the rest was imputed to RFI.

Laboratory analyses

Natural ^{15}N abundances ($\delta^{15}N$) in plasma and feed samples from all unpublished trials (n_indiv = 285) were analyzed using an isotope-ratio mass spectrometer (Isoprime Vision; Elementar France) coupled to an elemental analyzer (Vario cube; Elementar France). Analyses were performed in the same laboratory at INRAE (https://www6.clermont.inrae.fr/plateforme_exploration_metabolisme). For plasma preparation, 10 µL were pipetted into a small tin capsule, evaporated at room temperature for 24h and then the capsule closed before being loaded in the analyzer. Each plasma sample was analyzed in duplicate.

For dried and ground feed or TMR samples, between 2 and 12 mg were weighted (relative to their N content) and always analyzed in triplicate. Freeze dried muscle samples from #ID8 were analyzed by EA-irms in the stable isotope laboratory of the institute of Agricultural Science in Zurich, Switzerland (https://gl.ethz.ch/infrastructure/isolab.html). Individual isotopic data from published trials (ID#1 to ID#7) were recovered and included in the database.

In-house standards (glutamic acid or tyrosine) were included in each run every 10 samples to correct for possible time-variations in the analysis. Results were expressed using the delta notation according to the following equation:

$$\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1\,000,$$

where R_{sample} is the N isotope ratio between the heavier isotope and the lighter isotope (^{15}N /^{14}N) for the sample being analyzed, $R_{standard}$ the N isotope ratio of the internationally defined standard (atmospheric N_2, $R_{standard}$ = 0.0036765), and δ is the delta notation in parts per 1000 (‰) relative to the standard.
Samples were analyzed in duplicate and measurement errors for $\delta^{15}N$ analyzed in the in-house standards were always <1.1%CV (intra-assay). Nitrogen isotopic discrimination was calculated as the difference between $\delta^{15}N$

in plasma or muscle and that measured in the corresponding diet, the latter calculated by weighting the contribution of each ingredient to total dietary N. Plasma urea concentration was analyzed in samples from both published (n_study = 6) and unpublished (n_study = 5) trials in the same laboratory at INRAE through a spectrophotometric assay as described in Jorge-Smeding et al. (2021). No plasma samples were available from study #ID8 to conduct urea analysis.

Statistical analysis

All statistical analyses were performed using the R software (R core team, 2019). Two different approaches were explored for assessing the ability of the tested biomarkers to reflect the between-animal variability in feed efficiency as previously reported (Cantalapiedra-Hijar et al., 2018a). For both approaches we defined the between-animal variability as the variability observed within-CG, even though it is known to include a non-negligible and inherent part of experimental error. The two tested approaches aimed to either consider the between-CG variability in the model construction (mixed-effect model approach) or remove it before conducting simple regression analysis between feed efficiency traits and tested biomarkers (approach based on residuals).

Analysis of the relationship between feed conversion efficiency and either N isotopic fractionation or plasma urea

Simple and mixed effect models

As a first approach, both simple and mixed-effect models were tested to assess how well $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea correlated with FCE at the dietary treatment and individual levels. Thus, two different datasets were used in this analysis, the first containing dietary mean values for both variables and allowing us to strictly test treatment effects (i.e. diets and production context) and the second with all individual observations allowing us to assess between-animal variability (i.e within-CG relationship). For simple linear regression analysis, the lm function of R software was used while the nlme package of R (Pinheiro and Bates, 2000) was chosen for modeling the mixed-effect regression equations used. Models were built as follows:

Simple model: $Y_{ij} = \beta_0 + \beta_1 X_{ij} + \varepsilon_{ij,}$

Mixed-effect models: $Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_{ij} + \varepsilon_{ij,}$

where Y_{ij} is FCE and X_{ij} are values for either $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea concentration, β_0 and β_1 are the fixed effects for the intercept and the slope, respectively; the b_i are the random-effects of experimental factors (effect of either study alone, dietary treatment within-study or pen within- study [CG]) and assumed to be independent for different factors; and $\varepsilon_{ij,}$ are the identically distributed within-groups errors, assumed to be independent of the random effects for the mixed-effect models.

Different random structures (from simple to more complex models), were compared based on the Akaike Information Criterion (**AIC**; the lowest being best) and the Bayesian Information Criterion (**BIC**; the lowest being best). The random effects were tested on the intercept, slope or both. When information criterion statistics (AIC, BIC) were similar for two models, the log-likelihood ratio test criteria performed with the command ANOVA (model 0, model 1) in R was used to determine the best model. Random-effect structures were always compared (AIC, BIC) using the restricted-maximum likelihood method. Because the model accuracy depends on both the prediction model error (root mean square error of prediction; **RMSEP**) and the magnitude of the variability to be predicted, we extended the evaluation to other statistical criteria. Indeed, we calculated the ratio of RMSEP to the standard deviation of observations from the whole database (**RSR global**) or within-condition (**RSR condition**) to evaluate and compare models. The models were judged to be satisfactory if RSR ≤ 0.70 (Moriasi et al., 2007). The RMSEP from simple and mixed-effect model were obtained by using the chillR package in R while the RSR used the MuMIn package in R.

To test the effect of the breed on the explored relationship, a sub-dataset was created from those studies including at the same time early or intermediate vs late maturing breeds (ID#4, ID#5, ID#9, ID#11 and ID#13; n = 312). Animals were coded either as “Late” if the breed was a late-maturing pure or cross-bred, or alternatively “Early” (including breeds and cross-bred animals considered as early or intermediate maturing

breeds). The breed (Early vs Late) was included in the model as a fixed effect on the intercept and slope (biomarker × breed interaction) while the study and diet were considered as random.

Simple regression analysis after removing the between-CG variability

We regressed either RFI, or alternatively the between-animal variation in FCE, on the between-animal variation of both tested biomarkers regardless of the influences of experimental factors. The between-animal variability in FCE, $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea concentration was assessed once the random effect of CG was removed from raw values.

$$Y_{ij} = \beta_0 + CG + \varepsilon_{ij}$$

where Y_{ij} is either the observed FCE or $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea concentration, β_0 is the intercept, *CG* corresponds to the contemporary group effect, and ε_{ij} is the residual of the model reflecting the between-animal variation of FCE, $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea concentration.

This step was skipped for RFI, since it was by definition already free of the CG effect (i.e. model residuals are uncorrelated with any predictor included in the model [CG, ADG, $mBW^{0.75}$]). The resulting residuals were considered mainly due to the between-animal variation and unidentified sources of error (within-animal variation). If a relationship between RFI or FCE and the tested biomarkers was still significant once the raw values were corrected for effects of experimental factors, the ability of the biomarkers to capture between-animal variation of feed efficiency could be assessed.

Analysis of variance when selecting extreme animals in terms of feed efficiency

To further explore the ability of each biomarker to identify individual variability in feed efficiency, we selected the 20% most and 20% least efficient cattle in terms of either within-CG FCE values or RFI. A simple one-way ANOVA was then conducted to test whether the biomarkers values differed across these two contrasting groups of animals. Significant differences were set at P<0.05.

Combining biomarkers and growth performance data for feed efficiency prediction

Biomarkers and growth performance data were used in multiple regression analysis (lm function in the R software) to assess if their combination could strengthen the prediction of between-animal variation in feed efficiency. Variance inflation factor was obtained (car library in the R software) to evaluate the amount of multicollinearity among the independent variables included in multiple regression ($\Delta^{15}N_{animal\text{-}diet}$, plasma urea concentration and ADG [only for FCE]).

Results

Relationship between feed conversion efficiency and either N isotopic fractionation or plasma urea concentration

Simple and mixed-effect models

The overall negative correlation between FCE and $\Delta^{15}N_{animal\text{-}diet}$ across studies was moderate to high and regardless of using individual (r = -0.69, *P*<0.001; Figure 1A) or treatment mean values (r = -0.62, *P*<0.001; Figure 1C). However, a few within-diet relationships (Figure 1E) were not negative (3 out of 34) and only less than half (16 out 34) showed a significant negative relationship (*P*<0.05). Concerning plasma urea concentration, a low but still significant negative correlation was observed with FCE across studies by using both individual (r = -0.36, *P*<0.001; Figure 1B) or mean treatment values (r = 0.39, *P* = 0.04; Figure 1D). However, this same relationship evaluated within- treatment (Figure 1E), revealed that only 3 out of 28 responses were significantly negative (*P*<0.05) and 7 out of 28 showed a non-significant positive correlation.

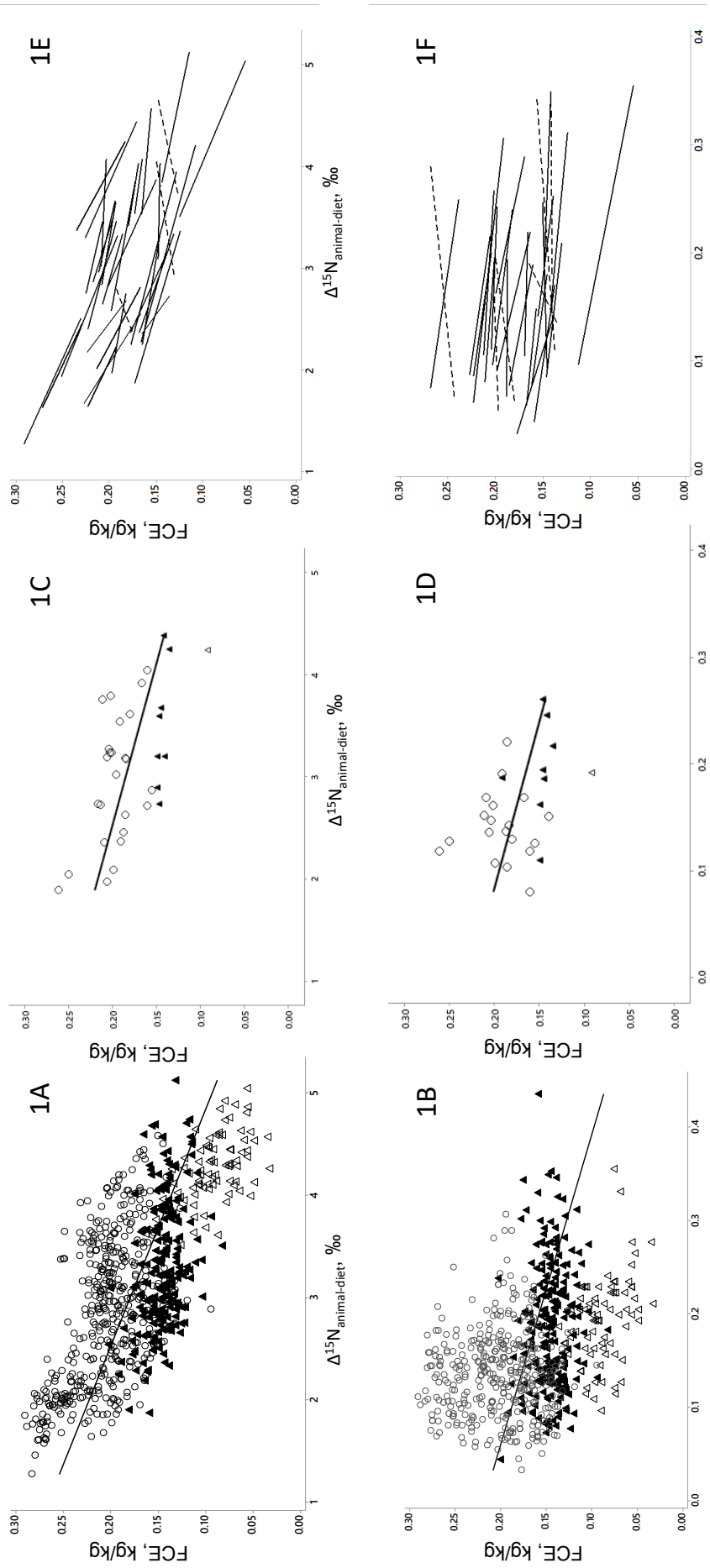

Figure 1. Relationship between feed conversion efficiency (FCE, kg/kg) and either N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$, ‰) or plasma urea concentration (g/L) in fattening beef cattle. On the left panels, simple linear regression across-studies using individual observations (1A: FCE = 0.31 – 0.043 × $\Delta^{15}N$ [n = 749; r = -0.69; *P*<0.001]; 1B: FCE = 0.22 – 0.30 × Plasma urea [n = 659; r = -0.36; *P*<0.001]) where open circles = young bulls, open triangles = beef heifers and closed triangle = beef steers. On the middle panels, simple linear regression using dietary treatment means (1C: FCE = 0.28 – 0.032 × $\Delta^{15}N$ [n = 34; r = -0.62; P<0.001]; 1D: FCE = 0.23 – 0.32 × Plasma urea [n = 28; r = -0.39; P=0.04]. On the right panels, simple linear regression within-treatment for $\Delta^{15}N_{animal\text{-}diet}$ (1E) and plasma urea (1F), where continuous line = negative relationship and dashed line = positive relationship. For $\Delta^{15}N_{animal\text{-}diet}$, 16 out of 32 within-treatment relationships were significant (*P*<0.05; 1E), whereas only 3 out of 28 were significant for plasma urea concentration (*P*<0.05; 1F).

Table 2 presents the simple and mixed-effect regression models of FCE on either $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea concentration. Concerning the N isotopic fractionation, all tested models (Eq. 1-5) showed a significant and negative response (slope) of FCE to the $\Delta^{15}N_{animal\text{-}diet}$ variation, but with different prediction errors and ability to identify the observed variability. Simple linear models had higher prediction errors (RMSEP) when based on individual observations (0.037 kg/kg) compared to treatment means (0.027 kg/kg). However, the model prediction error obtained from individual observations represented a lower proportion of the total observed FCE variability (RSR global = 0.727) compared to that based on treatment means (RSR global = 0.768). When using individual data, it was observed that $\Delta^{15}N_{animal\text{-}diet}$ was able to identify FCE variability once the experiment (Eq. 3), dietary treatment within-experiment (Eq. 4) or CG (Eq. 5) effects were removed from the relationship. However, no gain in model performance was observed when the pen effect, included in the CG, was added to the model beyond the study and treatment effect (Eq. 5 vs 4). In our conditions, models to predict FCE within-treatment or within-CG from $\Delta^{15}N_{animal\text{-}diet}$ were thus similar. The model prediction error of mixed-effect models, as well as its contribution to explain the observed total FCE variability (RSR global) was halved in comparison to simple models (Eq. 2 vs Eq. 3-5; Table 2). However, because the observed FCE variability logically decreases when shifting from a global to a within-condition level (i.e. when the variability from experimental factors is present or excluded, respectively), the lower prediction error obtained with the best mixed-effect model (0.016 kg/kg) did not translate to a greater ability to identify the observed FCE variability within that particular condition (RSR condition = 0.846). Based on a cut-off of <0.70 for RSR, optimal models for predicting total FCE variability were only those based on mixed-effect models from individual observations (Eq. 3-5). However, these same models had RSR condition > 0.70, meaning that they are not completely optimal for discriminating individuals reared in similar conditions (within-study, within-treatment or within-CG) but could rather be used to predict differences in animal FCE across-studies.

Models based on $\Delta^{15}N_{animal\text{-}diet}$ were always better in terms of RMSE, AIC/BIC and RSR than those based on plasma urea concentration. Although the response of FCE to plasma urea concentration was significant across studies for dietary treatment means or individual observations (Eq. 6-7), there was no significant relationships within-study (Eq. 8). Surprisingly, very weak but still significant relationships were found between FCE and plasma urea concentration within-treatment (Eq. 9) and within-CG (Eq. 10). Likewise, for $\Delta^{15}N_{animal\text{-}diet}$, no gain in model performance was found when adding the pen in addition to treatment effects. When breeds were categorized as early vs late maturing breeds from a sub-dataset, it was observed that the slope of the best model to predict FCE from $\Delta^{15}N_{animal\text{-}diet}$ (Eq. 4) tended to be higher (+0.005; P = 0.06) in Late vs Early maturing breeds while similar (P = 0.53) in the best prediction model from plasma urea.

Graphic presentation (Figure 2 and 3) of the best models for $\Delta^{15}N_{animal\text{-}diet}$ (Eq. 4; Table 2) and plasma urea concentration (Eq. 9, Table 2) did not reveal major violations for the assumptions of normality, homoscedasticity and independence.

Linear models and Anova after removing the between-CG variability

When the between-CG effect (pen within treatment and study) was removed from both feed efficiency and biomarker values, the relationship between feed efficiency traits (FCE or RFI) and either $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea concentration was always significant (P<0.01) but with different fits depending on the trait and biomarker (Figure 4).

Table 2. Simple linear and mixed-effect regression models of feed conversion efficiency on the N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) or plasma urea concentration using either dietary treatment means or individual observations from different fattening cattle production systems

Item	Equation	Intercept	Slope	RMSEP[1]	RSR global[2]	RSR condition[3]	R^2	AIC[4]	BIC[4]
$\Delta^{15}N_{animal\text{-}diet}$, ‰									
Treatment means, n = 34									
Simple linear model	1	0.283**± 0.023	-0.032** ± 0.007	0.027	0.768		0.39	-126	-122
Individual observations, n = 749									
Simple linear model	2	0.310**± 0.005	-0.043** ± 0.002	0.037	0.727		0.47	-2789	-2775
Mixed-effect models									
Study random effect	3	0.273**± 0.020	-0.032** ± 0.005	0.018	0.363	0.882	0.31	-3788	-3760
Treatment within study random effect[$]	4	0.277**± 0.018	-0.033** ± 0.004	0.016	0.325	0.846	0.33	-3826[$]	-3784[$]
Late vs Early maturing breeds[#]		+0.013±0.007[NS]	-0.005[†]± 0.002						
Contemporary group random effect	5	0.277**± 0.018	-0.033** ± 0.004	0.016	0.323	0.840	0.33	-3820	-3779
Plasma urea, g/L									
Treatment means, n = 28									
Simple linear model	6	0.224**± 0.025	-0.304* ± 0.150	0.033	0.912		0.15	-95.0	-91.2
Individual observations, n = 659									
Simple linear model	7	0.218**± 0.005	-0.301* ± 0.031	0.048	0.935		0.13	-2101	-2088
Mixed-effect models									
Study random effect	8	0.176**± 0.010	-0.019[NS] ± 0.028	0.021	0.406	0.982	0.00	-3114	-3087
Treatment within study random effect[$]	9	0.188**± 0.007	-0.066* ± 0.026	0.019	0.374	0.969	0.01	-3140[$]	-3112[$]
Late vs Early maturing breeds		+0.003±0.005[NS]	-0.019[NS] ± 0.002						
Contemporary group random effect	10	0.193**± 0.007	0.059* ± 0.026	0.019	0.369	0.956	0.01	-3073	-3046

[1]RMSEP = residual mean square error of prediction when comparing observed and predicted values (package chillR in R)

[2]RSR global = Ratio of the RMSEP (model prediction error) to the standard deviation of feed conversion efficiency observed in the whole dataset (across-study variability). The lower the better.

[3]RSR condition = Ratio of the RMSEP (model prediction error) to the standard deviation of feed conversion efficiency observed either within-study, or within-diet and study or within-CG. The lower the better

[4]AIC = Akaike information criterion (the lower the better); BIC = Bayesian information criterion (the lower the better)

[$]Best random structure model based on AIC/BIC criteria and the log-likelihood ratio test ($P < 0.05$).

[NS] Non significant (P>0.05) ; [**]$P \leq 0.001$; [*]$P \leq 0.05$; [†]$P \leq 0.10$.

[#]From a sub-dataset containing 312 observations from studies including both early or intermediate and late maturing breeds

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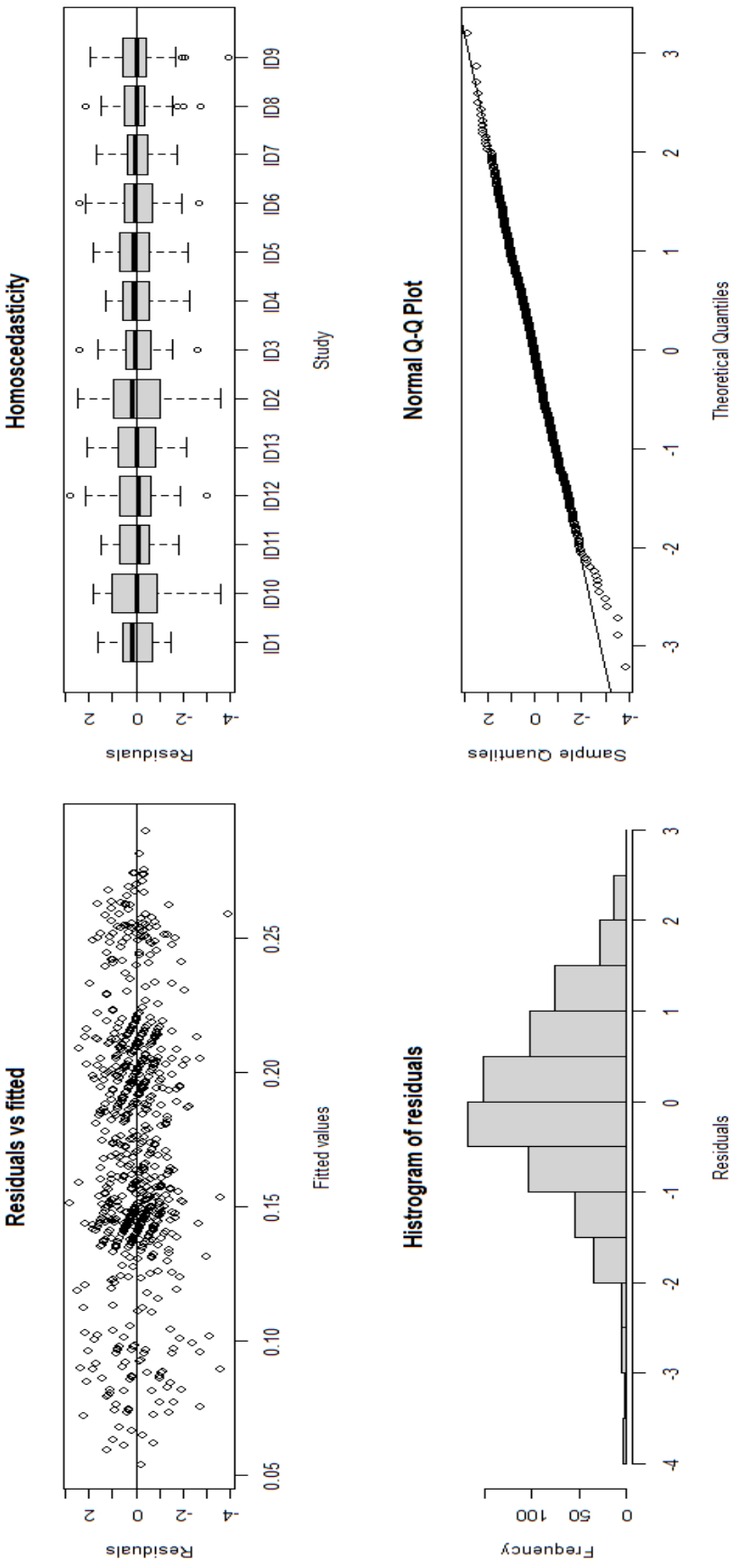

Figure 2. Model validation for normality, homogeneity and independence assumptions from N isotopic fractionation as a biomarker of feed conversion efficiency (Equation 4 in Table 2).

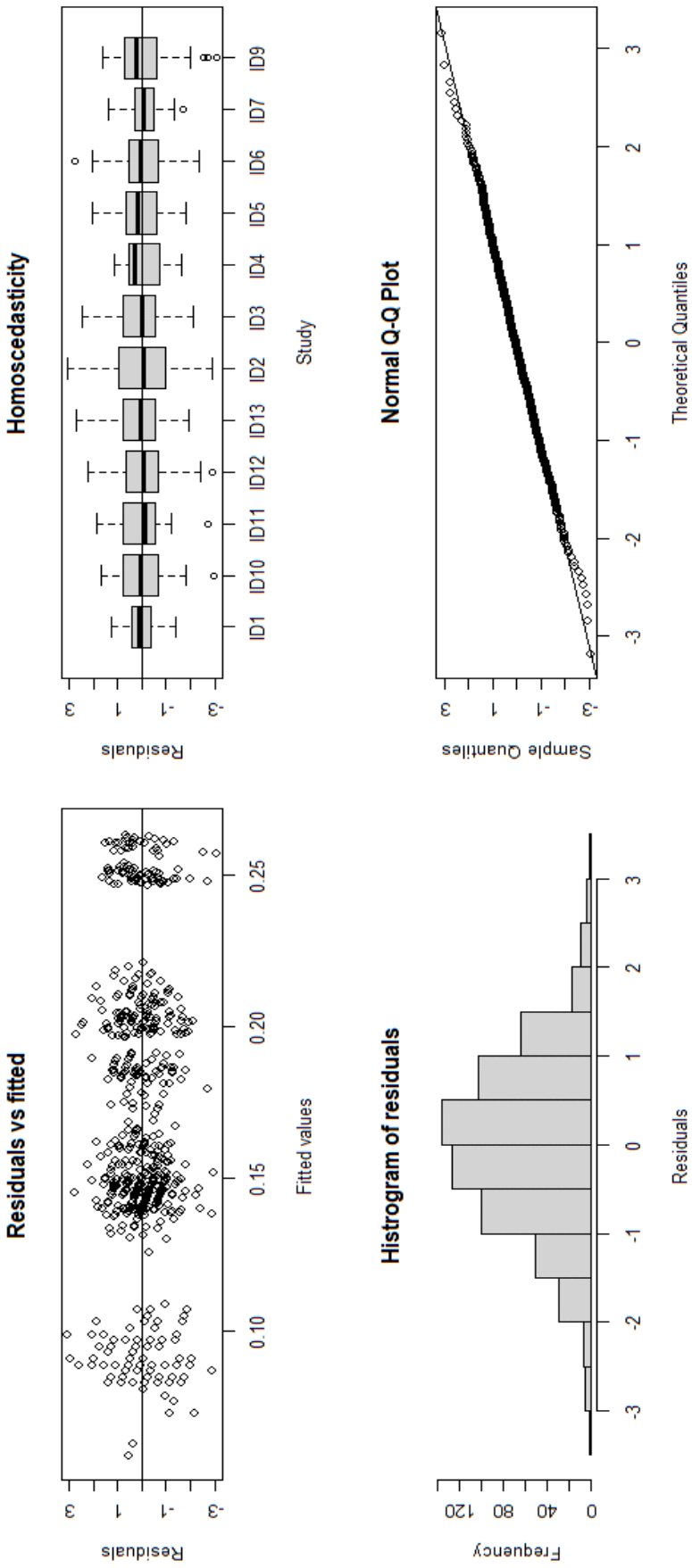

Figure 3. Model validation for normality, homogeneity and independence assumptions from plasma urea concentration as a biomarker of feed conversion efficiency (Equation 10 in Table 2).

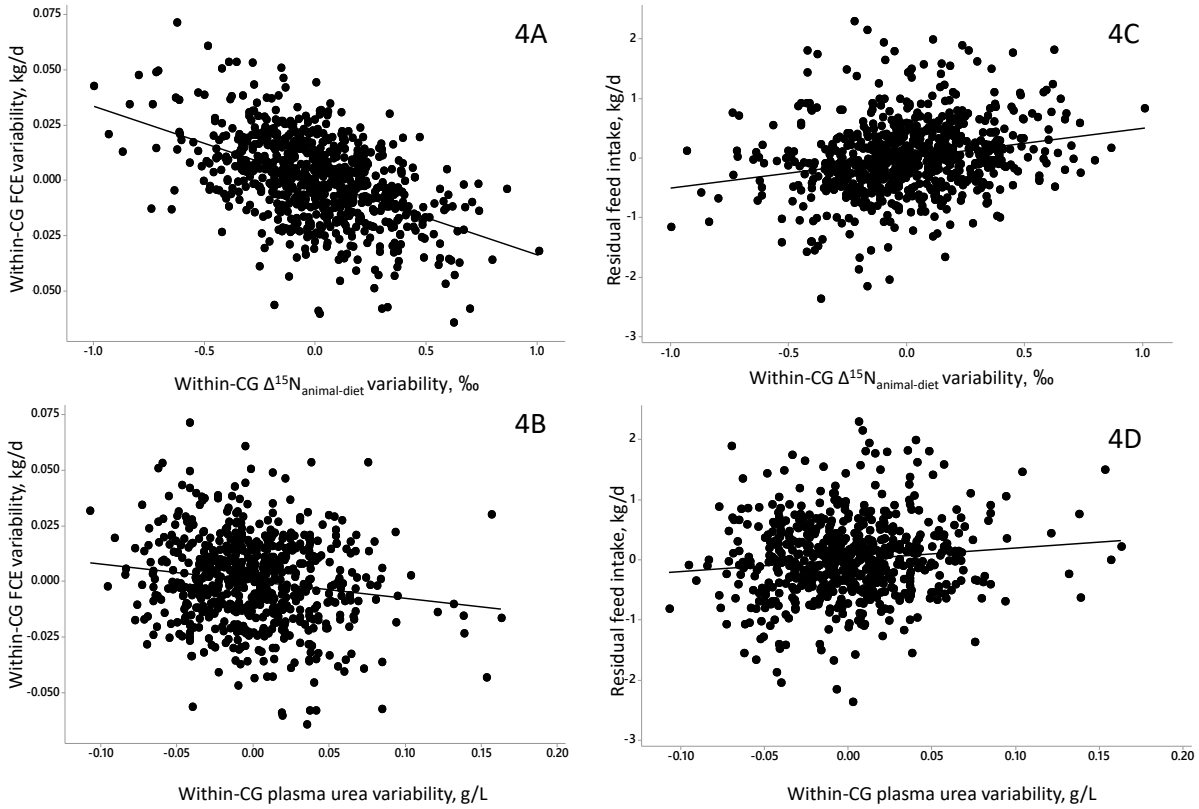

Figure 4. Relationships between feed efficiency traits (feed conversion efficiency; FCE [4A, 4C] and residual feed intake, RFI [4B, 4D]) and either N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$, ‰) or plasma urea concentration (g/L) in fattening beef cattle when variability across contemporary groups was previously removed (within-CG variability analysis). Equations (intercept not different from 0): (4A) Y = -0.034X (n = 749; r = -0.50; *P*<0.001); (4B): Y = -0.077X (n = 659; r = -0.15; *P*<0.001); (4C) Y = 0.50X (n = 748; r = 0.23; *P*<0.001); (4D): Y = 1.93X (n = 659; r = 0.11; *P*=0.003).

On average, greater model fits were achieved with $\Delta^{15}N_{animal\text{-}diet}$ compared to plasma urea and for FCE than for RFI. From higher to lower model fits were the regressions of FCE on $\Delta^{15}N_{animal\text{-}diet}$ (r = -0.50; *P*<0.001, Figure 4A), RFI on $\Delta^{15}N_{animal\text{-}diet}$ (r = 0.23; *P*<0.001, Figure 4C), FCE on plasma urea (r = -0.15; *P*<0.001, Figure 4B) and RFI on plasma urea (r= 0.11; *P*=0.003, Figure 4D). Although the two tested biomarkers were significantly correlated to feed efficiency traits at the individual level when using the whole dataset (Figure 4), their ability to discriminate the top 20% highest and lowest individuals within-CG in terms of feed efficiency varied (Figure 5). Indeed, both $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea concentration succeeded to discriminate the most extreme animals within-CG in terms of FCE (Figure 5A and 5B; P<0.001 and P=0.002), but only $\Delta^{15}N_{animal\text{-}diet}$ was significantly different (P<0.001) for extreme RFI animals (Figure 5C).

Combining performance and biomarkers to predict between-animal variability in feed efficiency

The within-CG variability of ADG itself explained 39% of the within-CG variability in FCE (Table 3). None of the tested biomarkers explained as much of the between-animal variability in FCE as ADG (25% and 2% for $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea, respectively). However, the combination of ADG and $\Delta^{15}N_{animal\text{-}diet}$ increased the explanation of FCE variability up to 51% with no further gain when adding plasma urea as a third variable. For RFI, the variance explained by both biomarkers was very low (5% and 1% for $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea, respectively) and the apparent slight gain when plasma urea was combined with $\Delta^{15}N_{animal\text{-}diet}$ (6%) was not significant (*P* = 0.06).

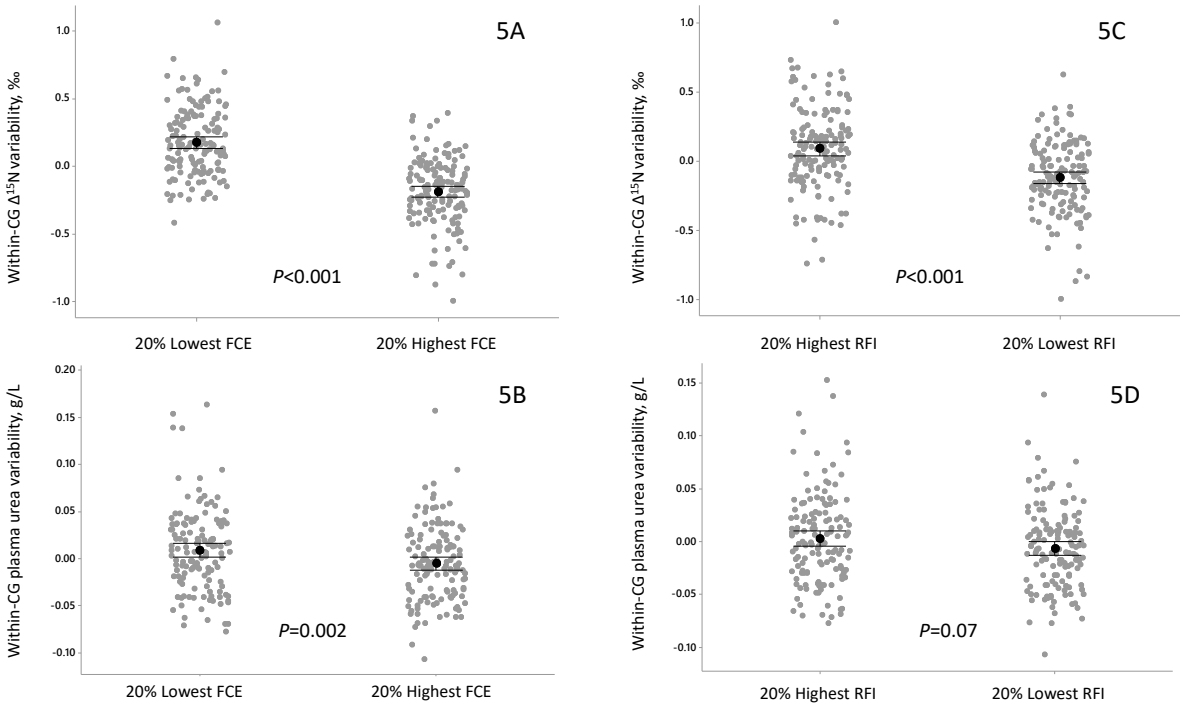

Figure 5. Within contemporary group values for N isotopic fractionation ($\Delta^{15}N$) and plasma urea concentration in the top 20% higher and lower efficient animals within contemporary group according to feed conversion efficiency (on the left; 5A and 5B) or residual feed intake (on the right; 5C and 5D).

Table 3. Simple and multiple linear regression for predicting within-contemporary group variability in feed conversion efficiency (FCE) and residual feed intake (RFI) from N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$), plasma urea concentration (PU) and average daily gain (ADG) in fattening cattle[1]

				P-value			
FCE	n	R^2	RSE[2]	ADG	$\Delta^{15}N$	PU	VIF[3]
ADG	749	0.39	0.015	<0.001			
$\Delta^{15}N_{animal\text{-}diet}$	749	0.25	0.017		<0.001		
Plasma urea	659	0.02	0.020			<0.001	
ADG + $\Delta^{15}N_{animal\text{-}diet}$	749	0.51	0.013	<0.001	<0.001		1.04
$\Delta^{15}N_{animal\text{-}diet}$ + Plasma urea	659	0.25	0.017	<0.001		0.06	1.03
ADG + $\Delta^{15}N_{animal\text{-}diet}$ + Plasma urea	659	0.51	0.014	<0.001	<0.001	0.07	1.03-1.09
RFI[3]							
$\Delta^{15}N_{animal\text{-}diet}$	749	0.05	0.60				
					<0.001		
Plasma urea	659	0.01	0.64			0.003	
$\Delta^{15}N_{animal\text{-}diet}$ + Plasma urea	659	0.06	0.63		<0.001	0.06	1.03

[1] The contemporary group effect was removed from all tested variables before analysis (excepting RFI already corrected)
[2] RSE = residual standard error
[2]ADG was not tested on RFI prediction since already included in the RFI calculation model
[3] Variance inflation factor (car library in R) measuring the amount of multicollinearity among the independent variables in a multiple regression model. Values higher than 5 may indicate multicollinearity.

Discussion

Exploring easy-to-use biomarkers enabling the identification and selection of superior animals in terms of feed efficiency is the first step towards assisting genetic selection and precision feeding through

biomarkers. Our findings confirm that N isotopic fractionation, a biomarker of NUE (Cantalapiedra-Hijar et al., 2018a), may explain between-animal variability in FCE and RFI to a greater extent than plasma urea concentration. Our results support the conclusions recently obtained by Guarnido-Lopez et al. (2021) about the potential of natural ^{15}N abundances in contrast to plasma urea, to predict at the individual level feed efficiency variability in young bulls reared in similar conditions. Furthermore, our results were obtained from more diverse cattle production systems across Europe, using different diets and breeds, which reinforces its potential to be applied in commercial farms. In addition, because our ^{15}N natural abundance in animal proteins were corrected by the isotopic signatures of the corresponding diets, unlike in our previous study (Guarnido-Lopez et al., 2021), we proved that $\Delta^{15}N_{animal\text{-}diet}$ may also reflect the FCE changes promoted by diets and thus serving to compare farms with different production conditions or to rank different dietary strategies in terms of FCE.

The ability of N isotopic fractionation vs plasma urea to reflect dietary treatment and between-animal variation in feed efficiency

From a theoretical point of view, individuals that better transform feed intake into growth should also present higher NUE (i.e. N retention to N intake ratio) compared to those with lesser feed efficiency. This is because on one hand, DM intake and N intake correlate positively at the individual level even if feed sorting occurs (Dykier et al., 2020). On the other hand, BW gain and N retention are highly correlated since protein and the associated water retention may account for 55-75% of the BW gain in growing-fattening cattle (estimations from values reported in Geay, 1984 and Cantalapiedra-Hijar et al., 2020a). The theoretical association between feed efficiency and NUE has been empirically confirmed in dairy cows with contrasted RFI values (Liu and VandeHaar, 2020) and in beef cattle ranked according to FCE (Nasrollahi et al., 2020). Thus, it can be reasonably argued that biomarkers of NUE could equally work for identifying feed efficiency variation in cattle. In this regard, we tested two proposed NUE biomarkers for ruminants: N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet;}$ Cantalapiedra-Hijar et al., 2018a) and blood plasma urea (Kohn et al., 2005). Our results showed that while both biomarkers could reflect FCE variation across different diets or production conditions, only $\Delta^{15}N_{animal\text{-}diet}$ seemed adapted to identify between-animal variability of FCE and, to a lesser extent, RFI variation.

According to the model performances obtained when using treatment mean values, our results indicated that $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea concentration could significantly distinguish two dietary treatments or production conditions (farms or studies) in terms of FCE if they differ by more than 0.10 kg/kg and 0.13 kg/kg, respectively (±1.96 × RMSEP at the 95% CI). This means for instance that the mean FCE obtained in the only trial conducted with growing heifers in our dataset (ID#1; mean FCE = 0.09; mean $\Delta^{15}N_{animal\text{-}diet}$ = 4.24‰) can be significantly discriminated at 95%CI from that observed in several other studies (ID#2, ID#3, ID#7, ID#8, ID#9 and ID#12; mean FCE = 0.21 kg/kg; mean $\Delta^{15}N_{animal\text{-}diet}$ = 2.76‰) when using $\Delta^{15}N_{animal\text{-}diet}$ (Table 1). Likewise, the production context promoting the highest FCE in young bulls in our dataset (#ID9; mean FCE = 0.26 kg/kg; mean $\Delta^{15}N_{animal\text{-}diet}$ = 1.95‰; mean ADG = 1.78kg/d) can be significantly discriminated from other trials using young bulls or steers (ID#4, ID#5, ID#11 and ID#13; mean FCE = 0.14 kg/kg; mean $\Delta^{15}N_{animal\text{-}diet}$ = 3.43‰; mean ADG = 1.53 kg/d) in a situation where the mean ADG did not differ too much between these contrasted conditions (+16%) compared to FCE (+85%). As expected, the number of studies that can be significantly discriminated in terms of FCE when using plasma urea was much lower because of its higher interval prediction error (+30%). Although NUE and FCE usually change proportionally at the individual level, this may not always occur across diets. In this regard, FCE remained unchanged in some beef cattle experiments despite use of different dietary N levels (Cortese et al., 2019; Da Silva et al., 2016). Moreover, high- vs low-N diets have been associated with higher FCE in some beef cattle feeding trials (Gabler and Heinrichs, 2003; Meneces et al., 2016; Cantalapiedra-Hijar et al., 2020a) despite the fact they are expected to decrease NUE (INRA, 2018). This may impair the ability of NUE biomarkers to identify FCE variation across dietary treatments if an excess or shortage of dietary protein supply in relation to requirements does not translate into feed efficiency variation or if it translates into an opposite trend (i.e. high FE-low NUE, low FE-high NUE). This is clearly illustrated in the trial conducted by Gabler and Heinrich (2003) with growing heifers where diets containing almost 20% CP promoted significantly higher FCE (0.23 kg/kg vs 0.21 kg/kg) but also higher plasma urea-N concentration (16.6 mg/dL vs 9.88 mg/dL) compared to diets containing 12% CP. The relationship between plasma urea concentration and FCE would be thus positive in that example (Gabler and Heinrich, 2003) rather than negative as we

have observed on average in our study (Figure 1D). The same holds true for $\Delta^{15}N_{animal\text{-}diet}$. Indeed, in one of the studies included in the present meta-analysis (#ID7; Cantalapiedra-Hijar et, 2020a) beef cattle fed diets formulated at 20% above protein requirement promoted on average higher FCE (0.198 kg/kg vs 0.186 kg/kg) but lower NUE (0.209 kg/kg vs 0.241 kg/kg) compared to diets formulated to meet protein requirements. On average, $\Delta^{15}N_{animal\text{-}diet}$ values in that study were in consequence higher with diets promoting greater FCE (2.80‰ vs 2.61‰), a positive relationship not observed on average across studies in the present work (Figure 1C).

Regarding the models based on individual observations, errors to predict FCE variations from $\Delta^{15}N_{animal\text{-}diet}$ or plasma urea (Eq. 4 and 9; Table 2) were compatible with significantly discriminating two individuals within-CG provided that their FCE differ by at least 0.06kg/kg and 0.08 kg/kg (±1.96 × RMSEP at the 95% confidence level), respectively. This minimal significant difference was only observed within-CG for about 11 and 6% of animals, respectively. These results are in close agreement with the conclusion obtained by Guarnido-Lopez et al. (2021) in young Charolais bulls reared in similar conditions, where the minimal difference needed for $\Delta^{15}N_{animal\text{-}diet}$ to significantly discriminate two individuals from the same CG was established at 0.05 kg/kg of FCE. According to Moriasi et al. (2007), a model can be judged to perform satisfactorily when the error represents less than 70% of the total observed variability (i.e. RSR<0.7). Only the mixed-effect models (vs. simple models) had RSR lower than this threshold, and thus they could be considered as optimal models to discriminate individuals reared under different production conditions on the basis of their FCE. However, if the aim is to compare individuals reared in the same conditions (e.g. within-CG), model performance in terms of RSR suggested that $\Delta^{15}N_{animal\text{-}diet}$ and plasma urea were not completely suitable to identify between-animal variability in FCE at the individual level (RSR condition >0.70 in Eq. 4 and Eq. 9). It is important to note that the model error inherently includes also the accumulation of errors from DMI and ADG measurements, the two pieces of information needed to calculate feed efficiency. To the best of our knowledge no measurement errors for ADG or FCE has been previously reported, but given the poor repeatability of these measurements in growing beef cattle (Kelly et al., 2010) it is expected that errors will be high compared to observed animal variance. Thus, the obtained prediction error from the present meta-analysis is expected to be highly influenced by the measurement errors associated to FCE and not only to the biomarker's inaccuracy. Further studies are needed to develop similar prediction models in very controlled experiments aiming at reducing as much as possible the source of error associated with FCE measurements. Although we did not reveal any relationship between the length of the feed efficiency test (from 56 to 259 days) and the correlation between biomarkers and feed efficiency (data not shown), the frequency of BW recording might have contributed to strengthen that correlation. As an example, the second highest correlation between FCE and $\Delta^{15}N_{animal\text{-}diet}$ was found (r = -0.66 within-CG) in the only experiment where BW was recorded every week (#ID13) rather than fortnightly or every 4 weeks (average r =-0.50 within-CG; Figure 4). Increasing the frequency for BW records has been demonstrated to improve the accuracy of feed efficiency measurements, especially in those tests using shorter durations (Archer et al., 1997). Interestingly, Archer et al. (1997) indicated that the accuracy of feed efficiency measurements could be mainly determined by accurate ADG measurements rather than intake. We hypothesize that the remaining high error for predicting FCE at the individual level with the tested biomarkers might be, above all, a consequence of the high measurement errors associated with BW gain measurements.

Our results indicate however that $\Delta^{15}N_{animal\text{-}diet}$ is still a powerful biomarker for discriminating a group of extreme animals in terms of FCE or RFI when comparing the 20% highest and lowest efficient animals within-CG (Figure 5A and 5C). This finding suggests that $\Delta^{15}N_{animal\text{-}diet}$ (and to a much lesser extent plasma urea) could be used within a herd to form groups of animals for precision feeding strategies, in such a way that the highest and lowest individuals in terms of $\Delta^{15}N_{animal\text{-}diet}$ could be assigned to receive diets with, for example, lower and higher CP content, respectively. In this regard, economic and environmental simulations in pigs genetically divergent for RFI demonstrated that high and low CP diets were more suitable for efficient and inefficient animals, respectively, than a common standard CP diet (Soleimani et al., 2021). Alternatively, the group of animals with the highest $\Delta^{15}N_{animal\text{-}diet}$ values (i.e. higher likelihood to have high RFI values) could be assigned to a precision feed restriction (Meir et al., 2019; Fischer et al., 2020) or to diets with a higher forage to concentrate ratio (Meir et al., 2021). These two promising dietary interventions are expected to lower feed N intake (compared to ad-libitum feeding or diets with lower forage to concentrate ratio) and have been proven to improve feed efficiency of dairy cows previously

ranked as RFI inefficient (Fischer et al., 2020; Meir et al., 2021). For precision feeding purposes further studies should evaluate first whether $\Delta^{15}N_{animal\text{-}diet}$ measured before weaning are correlated with differences in performance measured post-weaning, so as to serve as an early indicator of what kind of diets will match better (e.g. different N levels) with the post-weaning potential of animals.

The superior ability of $\Delta^{15}N_{animal\text{-}diet}$ vs plasma urea concentration to identify between-animal variability in feed efficiency (FCE and RFI) has been extensively discussed by Guarnido-Lopez et al. (2021). Advantages of $\Delta^{15}N_{animal\text{-}diet}$ vs plasma urea would include: i) the stability in daily values irrespective of the sampling time (i.e. no postprandial changes because of a relatively slow turnover rate), ii) little or no modifications by renal urea reabsorption or clearance rate and iii) its potential ability to also capture modifications in the rate at which tissue proteins are renewed (Remien, 2015), a true determinant of between-animal variation in feed efficiency (Cantalapiedra-Hijar et al., 2018b). Among its drawbacks it can be cited that: i) diet composition should be stable across a relatively long duration (i.e. FCE becomes unpredictable when diet composition changes), ii) the analysis by EA-irms is relatively time consuming (≈ 25 samples/day in duplicate) and expensive (≈ 15.00 €/sample in duplicate in France) and iii) representative samples of diets should be collected and analyzed if different feeding conditions or farms are being compared. A recent study found that rapid analysis by near-infrared reflectance spectroscopy may estimate N isotope composition in animal tissues with a relatively high accuracy (Ancin-Murguzur et al., 2021) opening the door to more throughput and cheap methods for predicting feed efficiency from equations herein proposed.

Interestingly, the response of FCE to $\Delta^{15}N_{animal\text{-}diet}$ variation at the individual level was almost identical with mixed effect models and simple regression based on residual (slope of -0.033 kg/kg [eq. 4; Table 2] and -0.034 kg/kg [Figure 4A]) and very close to the response of NUE to $\Delta^{15}N_{animal\text{-}diet}$ variations previously found by meta-analysis (-0.035 kg/kg; Cantalapiedra-Hijar et al., 2018a). Although caution is needed comparing different databases and studies, this could mean that on average both traits, FCE and NUE, are highly correlated at the individual level and so that one unit of change in $\Delta^{15}N_{animal\text{-}diet}$ is on average associated to similar changes in FCE or NUE (around 0.035 kg/kg). Given that Charolais, a late-maturing and lean breed, was the most representative breed in our database it can be expected that a change in body protein retention had a strong and proportional increase in body weight gain, explaining in part the proportionality between FCE and NUE changes at the individual level. This finding is supported by several previous studies showing that on average changes in FCE at the individual level followed proportionally those of NUE in fattening young Charolais bulls (Cantalapiedra-Hijar et al., 2015; Nasrollahi et al., 2020). For early-maturing breeds, where the contribution of fat deposition to body weight gain is higher, a measurement of the fat accretion assessed by ultrasound echography has been proven to provide additional strength to the relationship between FCE and $\Delta^{15}N_{animal\text{-}diet}$ (Meale et al., 2018). In this regard, when we attempted to evaluate the effect of the breed on the explored relationships it was observed that the response of FCE to changes in $\Delta^{15}N_{animal\text{-}diet}$ values tended to be greater in late vs early or intermediate maturing breeds. This finding would support the close link existing between $\Delta^{15}N_{animal\text{-}diet}$ and protein deposition in ruminants and the weaknesses that this biomarker could have to predict feed efficiency in slow growing animals or in animals retaining proportionally a greater amount of fat instead of protein. Further studies should confirm if different predictions models are needed for early vs late-maturing breeds.

Combining both biomarkers and growth data to strengthen feed efficiency predictions

The rationale for combining performance data (ADG), plasma urea and $\Delta^{15}N$ was the fact that each may relate to feed efficiency through common (moderate correlation between ADG and $\Delta^{15}N$; Figure 6) but also specific mechanisms. Adding different independent sources of variation could strengthen the prediction of the trait of interest (Negussie et al., 2017). Growth rate (ADG) is the single most important trait correlated with FCE as opposed to feed intake showing inconsistent relationships from one study to another (Arthur and Herd, 2012). Average daily gain is classically used by farmers and advisers as a proxy for animal feed efficiency when feed intake records are not available at the individual level on farm. In our conditions, ADG explained 39% of the total FCE variability within-CG, which was much higher than the percentage explained by any of the two tested biomarkers. Although our results might be interpreted as to better invest in BW recording systems on farm than in the analysis of these two biomarkers for phenotyping FCE, it must be remembered that biomarkers greatly reduce the time and cost to have an accurate estimate of traits.

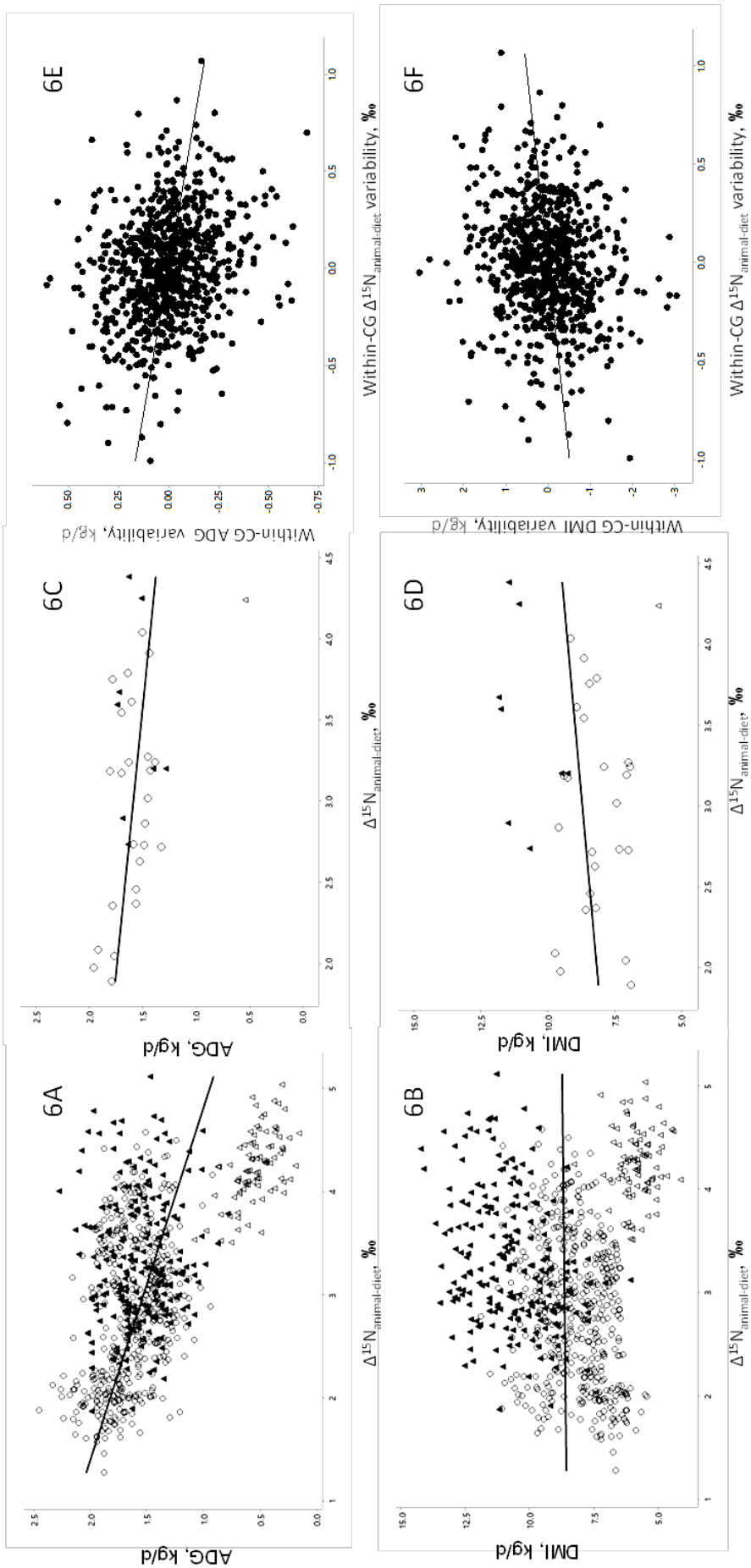

Figure 6. Relationship between N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$, ‰) and either average daily gain (ADG) or dry matter intake (DMI) in fattening beef cattle. (A-B) simple linear regression analysis using individual observations (6A: ADG = 2.51 – 0.33 × $\Delta^{15}N$ [n = 749; r = -0.59; P<0.001]; 6B: DMI = 7.94 + 0.26 × $\Delta^{15}N$ [n = 749; r = 0.1; P=0.04]) where open circles = young bulls, open triangles = beef heifers, closed circles = beef cows and closed triangle = beef steers; (C-D) simple linear regression using dietary treatment means (6C: ADG = 2.61 – 0.36 × $\Delta^{15}N$ [n = 34; r = -0.62; P<0.001]; 6D: DMI = 5.29 + 1.19 × $\Delta^{15}N$ [n = 34; r = 0.48; P=0.002]; (E-F) Simple linear regression when variability across contemporary groups was previously removed (within-CG variability analysis). Equations (intercept not different from 0): (6E) Y = -0.17X (n = 749; r = 0.24; *P*<0.001); (6F) Y = 0.51X (n = 749; r = 0.17; *P*<0.001).

Rather, adding $\Delta^{15}N$ in addition to ADG will significantly improve FCE predictions (R^2 from 0.39 to 0.51) indicating an additive effect on predictions. For RFI, no performance data were combined with biomarkers because of the expected lack of phenotypic correlation between RFI and growth performance data. Although both biomarkers are significantly (but weakly) related to RFI values, their combination did not improve predictions of RFI. This was somewhat expected, since both biomarkers share common metabolic pathways and plasma urea concentration values barely correlate with RFI (Guarnido-Lopez et al., 2021). Further studies are needed to assess the gain in prediction when combining $\Delta^{15}N$ values (stable over time) to other promising RFI biomarkers related to amino acid (Karisa et al., 2014; Foroutan et al., 2020) or lipid metabolism (Goldansaz et al., 2020) and more subjected to postprandial fluctuations and day-to-day variation.

Conclusions

This study demonstrated that $\Delta^{15}N_{animal\text{-}diet}$ can be proposed as a biomarker of feed efficiency to discriminate different production conditions or diets if they differ by at least 0.10 kg/kg of FCE. When the objective is to discriminate two animals from the same contemporary group $\Delta^{15}N_{animal\text{-}diet}$ may succeed provided they differ by at least 0.06 kg/kg of FCE. This minimal detectable difference (0.06 kg/kg of FCE) across individuals represent a limitation to predict FCE at the individual level and calls into question its use as tool for breeding programmes. However, our results highlight that $\Delta^{15}N_{animal\text{-}diet}$ can significantly discriminate groups of animals with contrasting FCE or RFI values (20% highest vs 20% lowest ranked animals). No gain in feed efficiency prediction was observed when combining both candidate biomarkers irrespective of the evaluated criteria. However, when ADG data was combined with $\Delta^{15}N_{animal\text{-}diet}$ values, the prediction of FCE at the individual level was strengthened compared to using only one of them, in which case ADG was the best single predictor. Our findings confirm that $\Delta^{15}N_{animal\text{-}diet}$ is related to the between-animal variability of feed efficiency in growing cattle to a greater extent than plasma urea concentration and suggest that it may be useful to form groups of animals for precision feeding when information about intake and body weight gain is lacking. More studies are warranted however to evaluate the usefulness of these two biomarkers to assist the genetic selection and the gain in prediction accuracy obtainable by combining them with other promising biomarkers of feed efficiency.

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Data, scripts and codes availability

The datasets and scripts used in this study are available at Zenodo (https://doi.org/10.5281/zenodo.5771504 and https://doi.org/10.5281/zenodo.5771531).

Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article. G. Cantalapiedra-Hijar is PCI Animal Science recommender.

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