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Abstract

Protein metabolism and body composition have been identified as major determinants of residual feed intake (**RFI**) in beef cattle fed high-starch fattening diets. This study aimed to evaluate if these 2 identified RFI determinants in beef cattle are the same across 2 contrasting silage-based diets. During two consecutive years, an 84-day feed efficiency test (Test A) immediately followed by a second 112-day feed efficiency test (Test B) was carried out using a total of 100 animals offered either one of two diets (either corn silage- or grass silage-based) over 196 days. At the end of Test A, the 32 animals most divergent for RFI (16 extreme RFI animals per diet, 8 low-RFI and 8 high-RFI,) were identified and evaluated during Test B for their i) N use efficiency (**NUE**; N retention/N intake) calculated either from a 10d-nitrogen balance trial or from estimations based on body composition changes occurring during the whole experiment (Test A and B; 196 days), ii) carcass and whole-body protein turnover rates analyzed through the 3-methyl-histidine urinary excretion and the N isotopic turnover rates of urine, respectively, and iii) body composition measured at the slaughterhouse at the end of Test B. Oxygen consumption was measured during Test B for the 100 animals by 2 GreenFeed systems. Irrespective of the diet, efficient RFI animals tended (P = 0.08) to improve their NUE when N retention was estimated for 196 days or when considering their lower urinary urea-N to total N ratio (P=0.03). In contrast, NUE calculated during the 10d-N balance showed no differences (P=0.65) across RFI groups suggesting that this method may not be suitable to capture small NUE differences. Efficient RFI individuals presented higher dressing percentage and muscle deposition in the carcass (P=0.003) but lighter rumen (P=0.001), and a trend for lower oxygen consumption (P=0.08) than inefficient RFI animals irrespective of the diet. Lower protein degradation rates of skeletal muscle and lower protein synthesis rates of plasma proteins were found in efficient RFI cattle but only with the corn silage-based diet (RFI x Diet; P=0.02). The higher insulinemia associated with the corn silage-based diet (P=0.001) seemed

to be a key metabolic feature explaining the positive association between protein turnover and RFI only in this diet. Feed N was more efficiently used for growth by efficient RFI animals regardless of the diet but lower protein turnover rates in efficient RFI animals was only observed with corn silage-based diets.

Key words: Beef cattle, feed efficiency, protein turnover, energy expenditure, nitrogen use efficiency

Implications

Selecting superior animals in terms of residual feed intake may improve beef farm profitability (less feed protein for the same performance) and decrease the environmental pollution (less N excretion for the same performance). However, protein metabolism, evaluated through the rates of muscle protein turnover, only explained differences in divergent residual feed intake cattle fed corn silage- but not grass silage-based diets. If further confirmed, this may imply that residual feed intake measured with high-starch or high-fiber diets is not exactly the same animal trait with potential consequences for beef cattle breeding programmes.

Introduction

Because of the increasing world demand for meat products, and the current economic and environmental context, there is a need to improve feed efficiency in livestock farming systems (MacLeod et al., 2018). This is particularly true for beef cattle which have a conversion rate of feeds into animal products that is considerably lower than that of other species (Mottet et al., 2017). Residual feed intake (**RFI**) is one of the preferred feed efficiency criteria for genetic selection because of its moderate heritability and uncorrelated response with animal BW and gain (Cantalapiedra-Hijar et al., 2018). However, some previous works highlighted that animals may change their RFI ranking across different diets (Durunna et al., 2011) especially when shifting from fattening energy-dense diets to diets rich in forages (Lahart et al., 2020) or inversely (Coyle et al., 2017). This would mean that breeding values for RFI derived with energy-dense diets may not be applicable to commercial grass-based beef production systems (Lahart et al., 2020). Although some hypotheses have been proposed to explain this re-ranking in feed efficiency, such as the contrasted ability of diets to express the full potential of animals (Keane et al., 2006) or the metabolic vs rumen regulations resulting from diets differing in carbohydrate nature (Lahart et al., 2020), little is known about the actual mechanisms. The study of biological determinants underlying RFI variations is thus of utmost importance to better understand the potential interactions between RFI and the type of diet fed to animals. Among the biological determinants of RFI, protein turnover, continual synthesis and degradation of body proteins not leading to changes in protein mass but associated only with protein renewal, has been proposed as a major one because of its associated energy expenditure (Richardson et al., 2004; Cantalapiedra-Hijar et al., 2018). However, only few studies have directly studied the relationship between RFI and protein turnover in growing or fattening cattle (Castro Bulle et al., 2007; Lines et al., 2014). This is probably due to the complex and

expensive techniques needed to study protein turnover by gold standard methods, especially in large animals as beef cattle (Cantalapiedra-Hijar et al., 2019).

Previous studies suggested an improved N use efficiency (**NUE**) in efficient RFI animals (Cantalapiedra-Hijar et al., 2020; Guarnido-Lopez et al., 2021), which could be partially explained by their lower protein turnover rates (McDonaugh et al., 2001; Cantalapiedra-Hijar et al., 2018). In addition, previous results from our team suggest that protein turnover could be related to RFI in a diet-dependent manner (Jorge-Smeding et al., 2021). We hypothesized that more efficient RFI animals will present lower protein turnover rates than inefficient ones specially with high-starch energy-dense diets. Therefore, the aim of this study was to evaluate the relationship between protein turnover and feed efficiency using a combination of gold-standard methods and new proxies in extreme RFI young bulls fed two contrasting diets (corn silage- vs grass silage- based diets). In addition, and because of their relationships with protein turnover and feed efficiency, we also evaluated body composition, NUE and energy expenditure in these extreme RFI animals. Some preliminary results in an abstract form have been published before (Guarnido-Lopez et al., 2021).

Material and methods

Animals, housing and experimental design

A total of 100 weaned 9-month-old purebred Charolais bulls were used in two consecutive years (n=50 in year 2019 and n=50 in year 2020). Bulls belong to a Charolais genetic program and their parents were known. At their arrival at INRAE the 100 animals (382 ± 41 kg BW and 259 ± 42 days old) were housed in an open shed with 2 pens and free stalls on a semi-mulched area composed of wood shavings. Pens were equipped with electronic feed gates and individual

automatic feeders (Biocontrol, Rakkestad, Norway). A total of 18 electronic feeders (9 by each pen) were used giving all animals, within their respective pen, free access to all electronic feeders. Each pen (n=2) was assigned to one of the two experimental diets, and animals were homogenously allotted to dietary treatments (with 25 animals per pen) ensuring a similar average and standard deviation in BW and age within diet. Diets were also balanced for the known genetic origin of bulls. During the experiment, which lasted 196 days for each year, an 84-d feed efficiency test (Test A) immediately followed by a second 112-d fee efficiency test (Test B) was carried out. Animal performances (Test A and B) and oxygen consumption (Test B) were measured on all animals while nitrogen balance, protein metabolism and body composition (all conducted in Test B) were only measured in the most divergent RFI cattle.

After 4 weeks of adaptation to the facilities and their experimental diet, all animals were evaluated during Test A for feed efficiency (day 0 to day 84); they averaged 414 ± 54 kg BW and 290 ± 42 days old at the beginning of the test. Results of the first feed efficiency test were used to rank and classify the animals as low RFI (efficient) and high RFI (inefficient); the 32 most RFI extreme animals were identified and selected (8 efficient and 8 inefficient for each diet per year). Then, during Test B, the extreme animals were measured for N balance (in two sub-groups per year, 8 animals per sub-group, the first sub-group between days 99 and 114, the second sub-group between days 114 and 129), protein metabolism (also evaluated in two subgroups, the beginning of the protein metabolism measurements coincided with the beginning of the nitrogen balance and the end coincided with the slaughter) and body composition at slaughter (day 196). Extreme RFI bulls were slaughtered on average after the same fattening duration (196 days), they averaged 698 ± 63 kg BW and 485 ± 42 day old.

Experimental diets

Experimental diets were based on either corn silage or grass silage and formulated with a forage to concentrate ratio close to 60:40 (Table 1). The grass silage was composed of permanent mountain grassland of Auvergne region in France. Composition of concentrates differed between diets, with wheat grains and soybean meal in the corn silage diet and the same ingredients plus beet pulp in the grass silage diet. Diets were distributed as total-mixed rations, and animals were fed individually and *ad libitum*. Both diets were iso-MP (≈ 83 g MP/ kg DM), but differed in their net energy concentrations (1.51 [grass silage diet] vs 1.66 [corn silage diet] Mcal/kg DM), calculated according to INRA (2018). The grass silage diet was rich in NDF (45% vs 33%, DM basis for grass and corn silage diets, respectively) while the corn silage diet was rich in starch (5% vs 33%, DM basis, same respective order).

Measurements and sampling

BW and intake

Animals were weighed on an electronic scale at 1400 (always in a non-fasting state) on two consecutive days at the beginning and at the end of each feed efficiency test, and by simple weighing every two weeks from the arrival of the animals in the experimental facilities until slaughter. Individual DM intake was calculated as the fresh matter intake measured by automatic feeders multiplied by the DM content of the total-mixed ration analysed five times (Monday to Friday) per week. The DM of ingredients, silages and whole total-mixed ration was analysed at the fattening facilities every day by dehydrating samples at 103°C during 24h.

Estimation of body composition during the first feed efficiency test

Body composition was estimated at the beginning (day 0) and at the end of Test A (day 84) using two different methods described in Cantalapiedra-Hijar et al. (2020). First adipocyte size was determined in subcutaneous fat sampled by biopsy and the whole-body lipid content was

predicted as in Garcia and Agabriel (2008). Second fat thickness was measured by ultrasound echography (Aloka Prosound 2 with a linear probe UST5820-5) in four different locations.

Nitrogen balance and associated urinary and plasma metabolites

Eight metabolism stalls developed at INRAE and manufactured by Chambron Gautier® (ZA Champ Lamet, Pont-du-Château, France) were used during Test B, which explains why each year the 16 extreme RFI animals were measured in two subgroups (8 animals per subgroup). Each animal remained 15 days in the metabolism stall, 5 days of adaptation and 10 days of intake measurement and fecal and urine collection. BW was individually recorded in two consecutive days at the beginning and at the end of the N balance.

For each animal, DM intake was determined as the difference between amounts of DM matter offered and refused. Daily representative fresh samples (200 g of each ingredient and of the whole total-mixed ration) and individual refusals (10% of total per day) were collected and stored at -20°C before N analysis. Total 24h-fecal excretion of each animal was collected at 0800, and representative fresh samples of feces were taken daily (10% of total per day). At the end of each collection period, all daily fecal samples were pooled by animal and stored at -20°C before chemical analyses (DM, N). Total 24h-urine was non-invasively collected using a harness attached to animals and connected to an electric vacuum pump that suctioned the urine into a 20-L flask acidified (pH<3) with 500-750 ml of 30% (v/v) H_2SO_4 to prevent N volatilization and microbial growth. Urine samples were collected daily at 0800 and at the end of the collection period samples were homogeneously pooled per animal (1% of the total daily excretion) and stored at -20°C before chemical and metabolites analyses (N, urea, creatinine and 3-methyl-histidine).

Before meal distribution, blood was sampled from each animal (n = 32) on the last day of its N balance period by coccygeal venipuncture into a 9 ml EDTA tube (BD vacutainer, Playmouth,

UK) and centrifuged (2500 x g during 10 min at 4°C). Plasma was stored at -20°C before urea and insulin determinations.

N isotopic turnover rates

We evaluated during Test B the protein turnover rates of the 32 extreme RFI animals through the N isotopic turnover method, which is based on the rate at which animal's proteins change their isotopic signatures after a small dietary isotopic switch (Cantalapiedra-Hijar et al., 2019). The determination of the N isotopic turnover consisted of measuring after a dietary N isotopic switch the N isotopic turnover rate in the plasma as a proxy of the fractional synthesis rate of hepatic export proteins and in the urine as a proxy of whole-body protein degradation rates (Cantalapiedra-Hijar et al., 2019). Animal proteins were enriched through oral administration of 60 mg/d/animal of ^{15}N-labelled urea (98% Atom percentage excess, Sigma-Aldrich, St. Louis, USA) before meal distribution and during the 14 days that preceded the start of animal adaptation to metabolism stalls (d-14 to d-1 in relation to the N balance trial). The artificial ^{15}N enrichment was stopped (i.e. dietary N isotopic switch) and blood and urine samples were obtained in kinetics from d0 onwards. Timing to start the dietary N isotopic switch and thus the first blood and urine samplings was aligned with the start of the adaptation period of animals during the N balance trial (d0) to ease the collection of the first urinary samples while the animal was on the metabolism stalls.

Plasma and urine samples were obtained over time to analyse the depletion of ^{15}N values measured as δ^{15}N (‰). Blood was sampled by coccygeal venipuncture in each extreme animal on 10 time points (d0, d3, d7, d11, d15 while animals where in the metabolism stalls and d21, d35, d49, d78 while they were in their respective pen). A last sampling was performed at the slaughterhouse (day 196). Blood was systematically sampled in the morning between 0800 and 1000, collected into a 9 ml heparin tube (BD vacutainer, Playmouth, UK), centrifuged (2500 g

196 x 10 min) before plasma separation and storage at -20°C. Urine samples were obtained from
197 each extreme animal on 13 time points. During the N balance period, urine samples (20 mL)
198 were taken early in the morning on d0, d1, d2, d3, d4, d7, d9, d11 and d14. When animals were
199 back in their pen, urine was collected manually after individual head blocking during 1 hour in
200 the morning on d17, d35, d70 and d142. A 20 mL sample was then transferred into a tube
201 containing 1 mL of 30% (v/v) H_2SO_4 and filtered through a 30 µm standard filter paper to
202 remove fine particles. The filtrate was stored at -20°C before analysis of ^{15}N enrichment.

203 *Measurements of body composition at slaughter*

204 Once all measurements were completed, extreme animals were slaughtered at the end of Test B
205 (from d196 onwards) at the INRAE experimental slaughterhouse of UE1414 Herbipôle Unit at
206 a rate of four animals per week (one efficient and one inefficient per diet) as soon as the first
207 animals reached approximately 720 kg of BW (corresponding to a target market carcass weight
208 of about 420 kg). Slaughter spread over 1 month (n=4 animals per week on June 2019 and June
209 2020) in order to keep the same average experimental duration between the two years. Each
210 week, all four animals were slaughtered on the same day (approximately 1 per hour) and the
211 order of sacrifice according to the different treatments (diet and RFI class) was changed in
212 order to balance these effects on all post-mortem measurements. Measurements of carcass traits
213 and visceral organ weights and dissection of the 6^{th} rib were conducted as previously described
214 (Meale et al., 2017).

215 *Oxygen consumption*

216 During Test B, measurements of O_2 consumption were done on all animals (day 84 to day 196)
217 through two coupled GreenFeed systems (GreenFeed® system, C-Lock Inc., Rapid City, SD,
218 USA), one in each pen, easily accessible for all animals in the pen. Measurements of O_2
219 consumption using the GreenFeed system are described in Supplementary Materials S1.

220

221 ***Laboratory analyses***

222 *Feed, experimental diet characteristics and N balance*

223 All feed samples from the whole fattening period and each N balance trial (2 per year) were
224 analyzed for DM, organic matter (**OM**), N, NDF, ADF and starch. Dry matter and OM
225 concentrations were determined by oven-drying (60°C and 72h) and subsequent incineration in
226 a muffle furnace at 550°C (NF V 18-101), respectively. Nitrogen concentrations were analyzed
227 in an elemental analyser (Rapid N cube, Elementar Analysensysteme Gmbh). Solid samples,
228 once dried, were introduced (200 mg) in capsules and liquid samples (urine) were introduced
229 by pipetting 250 µl in each capsule filled with cellulose. Crude protein was considered to be N
230 x 6.25. As described in Meale et al. (2017), all feed ingredients were analysed for their
231 enzymatic OM digestibility, and NDF and ADF contents while only concentrated feed were
232 assayed for their enzymatic CP degradability and starch content.

233 Feed values of individual ingredients were calculated according to INRA (2018) using the
234 Prevalim® module of the Inration V5® software. Nutritive values of experimental diets were
235 estimated using the Inration V5® software (https://app.inration-ruminal.fr/) from the ingredient
236 and chemical compositions of diets, and the digestive interactions which depend on the feeding
237 level (DMI in % of BW), the concentrate dietary proportion and the estimated rumen protein
238 balance (INRA, 2018).

239 *Plasma and urine metabolites*

240 Plasma ^{15}N abundance (**$\delta^{15}N$**) was analysed from bulk plasma samples. Ten µL of liquid
241 plasma was pipetted in a tin capsule and left to dehydrate at room temperature during 24h. In
242 previous experiments from our team, $\delta^{15}N$ analyses were conducted in plasma proteins (isolated
243 by precipitation with sulfosalicylic acid solution; 15µL into 300 µL of sample; 1 g/ml). Given

that more than 98% of total N in plasma is protein we decided to conduct the analysis directly on bulk plasma rather than in plasma proteins. Plasma samples from 4 animals were compared with both methods (bulk plasma vs plasma proteins) and similar results on both absolute $\delta^{15}N$ values and kinetics coefficients were obtained. The urinary $\delta^{15}N$ was analysed from the acidified urinary spot samples after being dehydrated in the same way as plasma samples. Dehydrated bulk plasma and liquid urinary samples were analysed for $\delta^{15}N$ by using an isotope-ratio mass spectrometer (Isoprime, VG Instruments, Manchester, UK) coupled to an elemental analyzer (EA Vario Micro Cube, Elementar, Germany) as described in Cantalapiedra-Hijar et al. (2020).

Urea concentrations were determined by two different methods, whether on plasma or urine samples. For plasma urea, analyses were conducted in duplicate through a colorimetric assay conducted in an automated analyzer (Arene 20XT, Thermo Scientific, Vaanta, Finland). The accuracy profile (NF V03-110: 2010) for concentrations ranging between 0.05 and 0.90 g/L yielded an average accuracy of 101% and a CV for replicates averaging 8%. For urinary urea, analyses were conducted using an enzymatic commercial kit (ABX Pentra; REF: A11A01641) on an automotive chemical benchtop analyser (ABX Pentra 400, Horiba Medical, Montpellier, France). Plasma insulin concentration was analysed by spectrophotometry using a microplate reader (Infinite® 200PRO NanoQuant, Tecan, Grödig, Austria) and an enzyme immunoassay kit (Mercodia Insulin Elisa, Mercodia AB, Uppsala, Sweden).

The urinary concentration of creatinine and 3-methyl-histidine were analysed by Bevital laboratory (Bergen, Norway, http://www.bevital.no/) through a gas chromatography combined with tandem mass spectrometry as described by Midttun et al. (2013).

Calculations

Feed efficiency and N use efficiency

Residual feed intake was calculated using individual average DM intake (**DMI**), average daily gain (**ADG**) and mid metabolic BW (**midBW$^{0.75}$**) for the first 84d-feed efficiency test. The ADG of each animal was calculated by regressing its BW over the time on test. Mid-test BW was predicted from the BW over time regression equations, and expressed at the power of 0.75. Feed conversion efficiency (**FCE**) was calculated as the individual ADG of animals divided by their correspondent DMI. The RFI model considered the effect of the year (2019-2020) and of the diet within the year, which is confounded with the pen. We tested if body composition measurements (fat thickness in each anatomical location or fat gain calculated through d84-d0) explained DMI variations in the RFI model. No effects of body composition resulted significant ($P \geq 0.45$) and were not included in the final RFI model. RFI was calculated as the difference between observed DMI and the DMI expected for a given midBW$^{0.75}$and ADG adjusted by the diet (pen) effect (D_e) as follows:

$$Y = \beta_0 + D_e + \beta_1 (MMBW) + \beta_2 (ADG) + e \quad \text{(Eq. 1)}$$

Where, Y is the observed individual DMI, β_0 is the intercept, D_e is the diet (pen) effect, β_1 is the regression coefficient for MMBW, β_2 is the regression coefficient for ADG, and e is the residual of the model or RFI. Each year, animals were ranked within each diet according to their RFI, the four highest (high RFI, non-efficient) and lowest (low RFI, efficient) ones for each experimental diet were selected, resulting in a total of 32 extreme bulls (n=8 efficient corn silage; n=8 efficient grass silage; n=8 inefficient grass silage; n=8 inefficient corn silage).

Nitrogen use efficiency was calculated as the retained N divided by N intake, the former either calculated from the N balance measurements (10 d), or from estimates of body protein gains over the whole experimental period (196 d). Calculations of N retention during the N balance trial or from body composition estimates at the beginning (subcutaneous fat biopsies) and at the

end (slaughter data) has been previously described (Cantalapiedra-Hijar et al., 2020) and are detailed in Supplementary Materials S2.

Protein metabolism

Post-diet switch $\delta^{15}N$ kinetics measured in plasma and urine were analysed by mono- and bi exponential models, respectively, as explained in Cantalapiedra-Hijar et al. (2019):

Mono-exponential model: $\delta^{15}N(t) = \delta^{15}N_{\infty} + (\delta^{15}N_0 - \delta^{15}N_{\infty}) \times exp^{-k \times t}$

Bi-exponential model: $\delta^{15}N(t) = \delta^{15}N_{\infty} + (\delta^{15}N_0 - \delta^{15}N_{\infty}) \times [p \times exp^{-k1 \times t} + (1-p) \times exp^{-k2 \times t}]$

where t (d) is the time since the ^{15}N diet-switch, $\delta^{15}N(t)$ (‰) is the pool $\delta^{15}N$ value at time t, $\delta^{15}N_0$ (‰) is the pool initial $\delta^{15}N$ value, and $\delta^{15}N_{\infty}$ (‰) is the asymptotic value of the pool after the animal has reached isotopic equilibrium with its basal diet (without ^{15}N-urea administration). In the mono-exponential model k (%/d) is the fractional N isotopic turnover rate of the pool which has been proposed as a proxy of the fractional protein synthesis rate (**FSR**) of hepatic plasma proteins (Cantalapiedra-Hijar et al., 2019). In the bi-exponential model, k_1 and k_2 (%/d) refers to the fractional N isotopic turnover rates of a fast and a slow turnover pool, the latter proposed as a proxy of the whole-body protein turnover rate (Cantalapiedra-Hijar et al., 2019). The coefficients of p and (1-p) refers to the modeled contribution of the fast and slow turnover pools to the whole N isotopic turnover rate.

In addition, urinary 3-methyl-histidine excretion measured during the nitrogen balance, was used as a proxy of the fractional degradation rate (**FDR**) of skeletal-muscle protein (Harris and Milne, 1981) as per the equation published by Castro-Bulle et al. (2007):

FDR (%/d) = ($[3\text{-methyl-histidine}]_{urine}$, μmol/L × urine volume, L/d) × (3-methyl-histidine_muscle, μmol)$^{-1}$,

where $[3\text{-methyl-histidine}]_{urine}$ represents the concentration of 3-methyl-histidine in the urine and 3-methyl-histidine_muscle is the total quantity of 3-methyl-histidine in the muscle, estimated from the skeletal-muscle protein mass and assuming 3.51 µmol of 3-methyl-histidine /g of muscle protein (Nishizawa et al., 1979). Skeletal-muscle protein mass was estimated from the protein retained in the carcass measured at the slaughterhouse (Supplementary Materials S3).

Statistical analysis

All statistical analyses were performed in the R software (RStudio Core Team, version 1.1.463, 2018), except for the N isotopic kinetics in plasma and urine, which were performed with XStat software (version 2020.3.1).

Treatment comparisons

To test the effects of the diet (corn silage- vs grass silage-based diets), the RFI group (low- vs high-RFI) and their interaction on all measured *in vivo* and *post-mortem* variables, a general linear model was run that included the year, the effect of diet, the RFI group, and their interactions as fixed effects (Year, Diet, RFI, Year x Diet, Year x RFI and Diet x RFI);

$$Y_{ijkl} = \mu + D_i + A_k + E_l + D_i \times A_k + E_l \times A_k + D_i \times E_l + \varepsilon ijkl$$

where Y_{ijkl} is the dependent variable for animal j, receiving diet i, in year k and belonging to RFI group l, µ is the overall mean; D_i is the fixed effect of the type of diet used (i = 1, 2), A_k is the fixed effect of the year (k = 1, 2), E_l is the fixed effect of RFI group as efficient or non-efficient (l = 1, 2), $D_i \times A_k$ is the interaction between the effect of the diet and year, $E_l \times A_k$ is the interaction between the effect of the RFI group and year, $D_i \times E_l$ is the interaction between the effect of the diet and the RFI group and εijkl is the residual term. The triple interaction was

also tested but as it was non-significant in all variables, we did not include it in the final model. Finally, and just in the case of slaughterhouse measurements and estimations we added the slaughter date as a fixed effect nested to the year (2 years, 4 different slaughter dates within each year) in order to account for differences in BW across time.

Mean values are reported as least square means with pooled SEM values. Some interaction terms were significant, but to simplify the presentation of results in tables, only the diet, RFI effects and their interaction (Diet x RFI) are shown. When the Diet x RFI interaction was significant, the means for the different RFI groups were compared using Tukey's significant difference multiple comparison. Effects were declared significant when $P \leq 0.05$, and a trend was considered when $0.05 < P < 0.10$.

Relationships between variables

Repeatability between RFI values determined for Test A (0-84 d) and Test B (84-196 d) was determined through Pearson's correlation (function COR in R). We also conducted a correlation matrix (Pearson's correlation) to evaluate within-diet the relationships between key variables analysed in this study (function COR in R).

Results

Although dietary formulation was the same in the two experimental years (2019 and 2020), differences in diet quality, especially in silages, differed slightly. The quality of silages was better in 2020 than in year 2019. As a result, the year effect was significant ($P < 0.05$) for animal performances, N balance, urinary N metabolites, the fractional depletion rate of the slow N turnover pool, the O_2 consumption, and measurements at slaughter and derived estimations. The year effect and its respective interactions with diet, RFI or the slaughter date (only for body composition) were thus considered in all statistical analyses but they are not shown in

tables in order to simplify results. None of the interactions between year × diet, year × RFI and year × diet × RFI were significant whatever the variable considered (P>0.05).

In vivo animal performances of extreme residual feed intake young bulls

As expected, RFI efficient (low RFI) bulls had on average a lower DMI (-8.7%, P=0.004) and a higher FCE (+8.49%, P=0.04) than non-efficient ones (high RFI) but a similar ADG (Table 2). Extreme RFI animals showed no differences (P≥0.26) in body composition (adipocyte size and ultrasound fat depth) neither at the beginning nor at the end of the Test A. Regarding the diet effect, animals fed the corn silage-based diet presented higher (P=0.001) DMI, ADG, FCE and consequently higher final BW (P=0.07), fat thickness (P=0.04) and adipocyte gain (0.009) than animals fed the grass silage-based diet, regardless of the RFI group.

Repeatability of DMI and RFI during the first and the second feed efficiency tests (Test A and B) was moderate to high (r>0.51 when analysing both diets together, Supplementary Table S1), confirming the consistency of RFI ranking during the whole measurement period. Numerically, repeatability of RFI was higher in extreme RFI animals fed the corn silage-based diet than in grass silage-fed animals (r=0.68 vs r=0.50, respectively).

Nitrogen partitioning, and plasma and urinary metabolites analysed in extreme residual feed intake young bulls

Because of a numerical difference in BW (+26 kg) at the beginning of the N balance trial between efficient and non-efficient individuals fed the grass silage-diet, we reported results by unit of BW (Table 3). Efficient (low RFI) animals had lower (-10%; P≤0.006) N intake, and fecal N flows than non-efficient (high RFI) animals irrespective of the diet. Conversely, urinary

384 N excretion differed between RFI groups according to the diet, the differences being higher in
385 corn silage vs grass silage fed animals (Diet x RFI; P=0.06). In addition, when N partition was
386 expressed in relation to N intake (including NUE), there were no significant differences
387 between extreme RFI individuals. Concerning the diet effect, animals fed the corn silage-based
388 diet presented higher ($P \leq 0.03$) N intake and urinary N excretion, but similar NUE and N
389 retention ($P \geq 0.16$) than animals fed grass silage-based diets.

390 During the N balance trial (Table 4), efficient RFI animals excreted less urinary urea ($P = 0.04$)
391 but had a greater urinary urea N to total N ratio than inefficient RFI animals ($P = 0.03$),
392 regardless of the diet, with a tendency for a greater RFI effect in animals fed the corn silage-
393 based diet (Diet × RFI; $P = 0.06$). The efficient RFI animals fed the corn silage diet also tended
394 to have lower insulin values than inefficient ones (Diet × RFI; $P = 0.08$). No effects of RFI
395 group was observed ($P > 0.05$) for plasma urea concentration and creatinine and 3-methyl-
396 histidine urinary excretion. In relation to the diet effect, plasma urea and insulin concentrations,
397 urinary 3-methyl-histidine excretion, ureic N over total urinary N and 3-methyl-histidine to
398 creatinine ratio were higher ($P \leq 0.01$) in animals fed the corn silage vs grass silage-based diet.

399

400 ***Protein metabolism and oxygen consumption***

401 Overall, protein metabolism parameters were not affected by the RFI group ($P>0.05$) when
402 considering both diets (Table 5). However, with the corn silage- but not the grass silage-based
403 diet efficient RFI animals showed both lower plasma fractional ^{15}N depletion rate and lower
404 skeletal-muscle fractional degradation rate (-10.2% to -14.7%) compared to their inefficient
405 counterparts (Diet × RFI; $P \leq 0.04$). A trend for a lower final ^{15}N enrichment of plasma (i.e.
406 natural ^{15}N abundance in plasma) in efficient vs inefficient RFI animals was equally observed
407 with the corn silage- but not the grass silage -based diet (Diet × RFI; $P = 0.08$). In addition, and

408 despite non-significant differences in the urinary fractional ^{15}N depletion rate of the slow N
409 turnover pool across RFI extremes (P>0.05), efficient animals showed numerically lower
410 whole-body protein FDR (-12.1%) than non-efficient RFI individuals only when fed the corn
411 silage-diet (Diet × RFI; P=0.38). The oxygen consumption per kg BW (Supplementary Table
412 S2), tended to be lower (-5%; P = 0.08) in RFI efficient animals.

413 Regarding the diet effect, animals fed the corn silage-based diet presented a higher (P≤0.03)
414 initial and final plasma ^{15}N enrichment, fractional ^{15}N depletion rate in plasma, urinary
415 fractional ^{15}N depletion rate in the slow N turnover pool and lower initial urinary ^{15}N
416 enrichment in the slow N turnover pool. Moreover, the estimated skeletal-muscle FDR was
417 higher (+6.7%) in RFI inefficient animals fed the corn silage-based diet only.

418

419 ***Post-mortem performances and body composition determined at slaughter***

420 Because the average fattening duration was similar across RFI groups, the BW before bleeding,
421 empty BW and the conformation score were similar (P>0.14) across RFI or diet groups
422 (Supplementary Table S3). However, the carcass weight and dressing percentage were higher
423 (+5% and +3.2%, respectively; P≤0.03) in efficient vs non-efficient individuals regardless of
424 the diet (Table 6). Only with the corn silage -based diet, a lower carcass fat score (-12.8%, Diet
425 × RFI; P=0.05) was noted for efficient RFI cattle. The weight of digestive contents was no
426 different between extreme RFI individuals or between diets (P>0.54). Organs and body parts
427 were reported per unit of empty BW in order to mitigate the differences in BW at slaughter
428 (Supplementary Table S3). Efficient animals had a lower proportion of head (-4.4%; P=0.02)
429 than non-efficient ones regardless of the diet, with similar proportions of feet (P=0.86) and skin
430 (P=0.21). They had a smaller (-8.4%; P=0.001) rumen-reticulum regardless of the diet, leading
431 to a tendency for a lower proportion of total internal organs than non-efficient (-3.5%; P=0.09).

Total internal and subcutaneous fat were not significantly different ($P \geq 0.12$) between extreme RFI animals, but tended (Diet × RFI, $P \geq 0.07$) to be lower (-15% and -33%, respectively) in efficient animals fed the corn silage-based diet. Regarding the diet effect, animals fed the grass silage-based diet had lower trimmed carcass fat ($P=0.03$) but larger ($P<0.05$) non-carcass parts (head, feet, testicles, liver, heart, kidneys and whole-digestive tract) than animals fed the corn silage-based diet.

The measured tissue composition of the 6th rib and estimated composition of carcass (tissues) and whole body (chemical) are shown in Table 6. Efficient RFI animals presented a higher proportion of muscle (+3.2%; $P=0.02$) and a lower proportion of bones (-9.7%; $P=0.02$) with a trend for a higher muscle to fat ratio (+24%) with the corn silage-based diet only (Diet x RFI; $P = 0.08$). Concerning the diet effect, animals fed the grass silage-based diet presented a lower proportion of muscle ($P=0.01$) and higher proportion of bones ($P=0.001$) in the 6th rib than animals fed the corn silage -based diet.

Body and carcass composition calculated from the 6th rib composition showed that efficient RFI animals tended to have a higher proportion of muscle (+1.5 percent units) and a lower proportion of fat (-1.2 percent units) in the carcass only when fed the corn silage diet (RFI × Diet; $P \leq 0.09$) with no differences in skeleton proportion ($P=0.12$). The chemical composition of the whole-body was not significantly different ($P \geq 0.24$) between extreme RFI animals. No diet effects were noted for the carcass tissue or whole-body chemical compositions ($P \geq 0.11$).

Gain in empty BW, protein and fat over the whole duration of the trial was similar ($P \geq 0.26$) between extreme RFI animals, but tended to be higher ($P<0.07$) for corn silage fed animals (Table 6). The NUE calculated from the total N gain tended to be higher (+10.6%; $P=0.08$) for efficient RFI animals regardless of the diet.

Discussion

Our results highlight that some mechanisms associated with RFI in fattening bulls were common to both diets, such as the NUE improvement, higher dressing percentage or lower oxygen consumption in efficient vs non-efficient RFI animals. However, the association between RFI and protein turnover rate was only observed with corn silage-based diets supporting the concept of some diet-specific metabolic pathways underlying RFI differences (Jorge-Smeding et al., 2021).

N use efficiency is improved in low residual feed intake animals, irrespective of the diet

From a mathematical point of view, efficient RFI animals should present higher NUE (i.e. greater N retention per unit of N intake) than non-efficient animals because of their lower N intake but similar body gain (Carmona et al., 2020) and protein retention (Castro-Bulle et al., 2007). From a biological point of view, higher NUE is also expected in RFI efficient vs non-efficient animals because the lower feeding level of efficient RFI animals should lead to lower fecal endogenous protein losses (INRA, 2018) and lower hepatic amino acid catabolism (Lobley et al., 1992), both entailing N efficiency processes (Calsamiglia et al., 2010).

The combination of different results from the present work suggests that efficient RFI cattle had a better NUE compared to inefficient ones irrespectively of the diet although statistically significance was not reached for all relevant variables because of methodological limits associated to some of them. When N retention was estimated for the whole fattening period (d0-d196) from slaughter data, efficient RFI cattle tended to have an improved NUE over inefficient ones for both diets. This improved NUE is supported by the lower urea-N to total N ratio in urine observed in efficient vs inefficient RFI cattle, indicating a lower partition of N intake towards urine resulting from lower hepatic AA catabolism (Lapierre et al., 2006) or

480 rumen ammonia absorption (Huntington and Archibeque, 1999) or both. Improved N gain at
481 the expense of urinary N loss was also measured in efficient RFI bulls by Guarnido-Lopez et
482 al., (2021) using the natural ^{15}N abundance as a NUE biomarker and a larger experimental
483 setup (n=600, including the present bulls).

484 By contrast, NUE calculated from the N balance measurements did not support a greater NUE
485 in efficient RFI animals despite a measurement period longer than the minimum recommended
486 duration (10 d vs 7 d; Firkins and Reynolds, 2005) and a moderate to high repeatability (r=0.77;
487 data not shown) of intake between the first RFI test (Test A; 0-84d) and N balance trial (Test B;
488 99-114d). We assume that the N balance method did not capture NUE differences between
489 extreme RFI individuals in beef cattle because of the high experimental errors associated to the
490 determination of N retention in growing animals (Martin, 1966). The N balance method is
491 largely used to compare N partitioning among dietary treatments (Rumsey et al., 1999) but
492 might not be suitable to detect small NUE differences across individuals fed the same diet. An
493 a posteriori power analysis from our own data revealed that 89 animals per group would be
494 needed for detecting a significant difference in NUE equal to or higher than 10% (i.e. the
495 observed size effect in the corn silage diet). Similarly several studies conducted in growing
496 beef cattle (Lines et al., 2014; Carmona et al., 2020) failed to demonstrate differences in NUE
497 across extreme RFI individuals when using the N balance method. In contrast, an improved N
498 balance and a greater NUE were measured in efficient RFI dairy cattle (Xie et al., 2019; Liu
499 and VandeHaar, 2020), despite an absence of significant differences in urinary or fecal N
500 excretions between extreme individuals (Xie et al., 2019).

501

502 ***Dressing percentage increases in low residual feed intake animals, irrespective of the diet***

503 The higher partition of N intake into gain noted in efficient RFI animals was associated with
504 heavier and leaner carcasses, as in Basarab et al. (2003). Indeed at similar BW, efficient RFI
505 animals presented a greater dressing percentage than their non-efficient counterparts, which
506 resulted from a higher carcass weight, a greater muscle to fat ratio in the carcass and a smaller
507 non-carcass part (essentially visceral organs and head) whatever the diet. We failed to capture
508 differences in the whole-body fat content across RFI groups, but Charolais breeds are among
509 the leanest breeds with the lower phenotypic and genetic association to carcass fat deposition
510 (Kelly et al., 2014) and differences among RFI groups might be too small to be detected. Our
511 results agree with previous studies using large datasets already showing that dressing
512 percentage is phenotypically and genetically higher in efficient vs non-efficient RFI Charolais
513 young bulls (Taussat et al., 2019). In addition, differences in the non-carcass part were only due
514 to smaller rumen-reticulum and smaller heads of efficient RFI individuals because of similar
515 amounts of digestive contents between RFI groups (Supplementary Table S3), confirming
516 results from Kenny et al. (2018) on visceral organs (digestive tract mainly). This point is
517 important because rumen size is known to vary with the feeding level (Basarab et al., 2003).
518 Therefore, current results suggest that the higher intake of inefficient RFI animals may result in
519 bigger rumens and thus in lower dressing percentage. Because of their high metabolic rate,
520 digestive organs may compete with peripheral tissues for AA (Lapierre et al., 2006) and thus
521 decrease the overall NUE as observed in the present study. Although our experimental design
522 does not allow to identify cause-effect relationships, our data may support the concept of a
523 biological link between the dressing percentage and NUE as previously reported in growing
524 ruminants (Fluharty et al., 1999) across diets.

525

526 ***Protein turnover but not oxygen consumption is related to residual feed intake in a diet-***
527 ***dependent manner***

Because of its high metabolic cost associated with non-productive functions, protein turnover rate has been suggested to be a biological mechanism underlying RFI variation in beef cattle (Richardson and Herd, 2004; Cantalapiedra-Hijar et al., 2018). In the present study, we found lower protein turnover rate in skeletal muscle of efficient RFI cattle but only when they were fed the corn silage-based diet. As discussed herein, this finding is closely associated with the observed differences in carcass composition across RFI groups and could be explained by the contrasted nutrient absorption expected across diets.

Carcass muscle deposition tended to be higher in efficient RFI bulls fed the corn silage-based diet only, as indicated by the muscle proportion measured in the 6^{th} rib or estimated in the whole carcass as well as the carcass fat score, all pointing to a trend for leaner carcass. The greater carcass muscle deposition in these animals may be explained by their lower skeletal muscle protein degradation rate estimated from the urinary 3-methyl-histidine excretion, providing that protein synthesis did not decrease in a similar or greater extent. Indeed, in the present study carcass muscle proportion and dressing percentage were both negatively correlated with the skeletal muscle protein degradation rate in corn silage- but not in grass silage-based diets (Supplementary Tables S4 and S5). Although we did not measure the fractional protein synthesis rate in this specific pool, our data suggest that both protein synthesis and degradation rates in carcass muscle were downregulated in efficient vs non-efficient RFI cattle, only when fed a corn silage-based diet, though to a different extent. First, in the corn silage-based diet the RFI difference observed for carcass muscle proportion (a proxy of the protein accretion rate) was much lower than the RFI difference found for the skeletal muscle protein degradation rate (2% vs 17%). This led us to think that protein synthesis rate may have also decreased, though to a lesser extent; otherwise the impact on carcass muscle proportion would have been greater. Second, the N isotopic turnover rate measured in plasma proteins, a proxy of the hepatic fractional synthesis rate of plasma proteins (Cantalapiedra-Hijar

et al., 2019) also slowed down in efficient RFI animals but only when fed the corn silage-based diet. Hepatic synthesis of export proteins generally decreases as nutrient supply decreases (Connell et al., 1997). Lower nutrient absorption is expected with the lower feed intake of efficient RFI animals, yet lower hepatic plasma protein synthesis in efficient RFI cattle was only observed in our study with corn silage-based diets. Moreover, the hepatic synthesis rate of export proteins, unlike that of the constitutive ones, may increase in response to insulin secretion in a similar way as the skeletal muscle does (Davis et al., 2001). According to Raj et al. (2004) there may exist a coordinated increase in the protein synthesis rate of hepatic plasma proteins in humans facilitated by the constant delivery of amino acids from muscle degradation rate while Sheffield-Moore et al., (2005) argued that there may exist a complex relationship and interdependence between the metabolism of the hepatic synthesized plasma proteins and skeletal muscle protein. Whether this coordinated response or cross-talking observed in specific physiological or pathological situations in humans also occur in ruminants remains to be elucidated. However, our data showed that despite of using different methodology a significant correlation was observed between plasma proteins synthesis and skeletal muscle protein degradation rates only in corn silage-based diets ($r = 0.47$; Supplementary Table S4), suggesting a lower protein synthesis rate in skeletal muscle of efficient RFI cattle fed the corn silage-based diet. With the available data, we hypothesize that both protein synthesis and degradation in carcass muscle were likely downregulated (i.e. lower protein turnover) in efficient RFI cattle when fed corn silage- but not grass silage-based diet. The likely greater decrease in protein degradation vs synthesis in these animals would support their higher carcass muscle accretion. As discussed in the review by Cantalapiedra-Hijar et al. (2018), from a theoretical point of view, the most economical way of achieving higher growth efficiencies would be by a reduction in the protein degradation rate rather than by an increase in the synthesis rate. Our data seems to support this theoretical concept only in animals fed the corn

578 silage-based diet since the greater growth efficiency of low-RFI animals (i.e. lower intake for
579 similar performances) is associated with lower protein turnover rate in carcass muscle. Others
580 studies using similar approaches did not observe however differences in protein turnover of
581 skeletal muscle across extreme RFI beef cattle (Richardson and Herd, 2004; Castro Bulle et al.,
582 2007; Gomes et al., 2012) even if animals were fed with energy-dense diets rich in starch.
583 Differences in breed (all of the above-mentioned studies used early-maturing breeds), age or
584 the extent of RFI divergences across extreme RFI animals could somehow explain this
585 disagreement. In contrast, the study by McDonaugh et al., (2001) found higher calpastatin
586 activity in muscle of efficient RFI beef cattle compatible with a lower protein degradation rate
587 in carcass muscle.

588 Given the significant contribution of skeletal muscle to the whole-body protein turnover
589 (Lobley, 2003) we would also have expected a lower whole-body protein turnover in efficient
590 RFI cattle fed the corn silage-based diet. However, our proxy for the whole-body protein
591 degradation rate (i.e. the ^{15}N depletion rate of the whole-body slow turnover pool modeled from
592 urine kinetics) only showed numerically lower values (-12%) in efficient vs inefficient RFI
593 cattle fed corn silage-based diets. Although this new proxy appears promising to discriminate
594 the whole-body protein turnover across diets differing in protein content (Cantalapiedra-Hijar
595 et al., 2019) or energy nature (data from the present experiment) it may not be accurate enough
596 to discriminate individuals fed the same diet and showing relatively small differences in the
597 whole-body protein turnover rates.

598 Logical questions arise on why the association between skeletal-muscle protein turnover
599 rates and RFI are only observed with diets based on starch (corn silage) but not on fiber (grass
600 silage). Experimental diets mainly differed in the nature and density of the energy (Table 1),
601 leading to a theoretical greater portal absorption of glucose (+91%) and of the propionate to
602 acetate ratio (+13.6%) in corn silage- vs grass silage-based diets as predicted from chemical

603 analysis and INRA equations (data not shown). Consequently, the expected RFI differences in
604 the absorption of glucogenic substrates may explain the greater insulin plasma concentration
605 observed in corn silage-based diets (Loncke et al. 2020). Protein synthesis is regulated by the
606 mammalian target of rapamycin, a pathway which is activated by both insulin and branched
607 chain AA signaling cascade (Laplante and Sabatini, 2009) and has been demonstrated to be
608 upregulated in tissues of efficient RFI beef cattle fed corn silage-based diets (Elolimy et al.,
609 2019). The greater insulin plasma concentration observed in animals fed the corn silage- vs
610 grass silage-based diet could have promoted a favorable metabolic context where the greater
611 amino acid absorption expected in inefficient RFI animals may have translated into higher
612 skeletal muscle protein synthesis through the mammalian target of rapamycin pathway. In this
613 regard, Jorge-Smeding et al. (2021) evaluated extreme RFI Charolais young bulls fed almost
614 identical diets than in present study and found that the combination of high plasma insulin and
615 branched-chain AA concentrations in inefficient RFI animals was observed in corn silage but
616 not in grass silage diets, a metabolic feature compatible with the expected increase in muscle
617 protein turnover rates in these animals. Finally, it can be acknowledged that, although non-
618 significant, BW differences across RFI groups fed the grass silage diet at the start of the
619 experiment (7% higher BW for efficient RFI animals at the start of Test A; Table 2) might
620 explain to some extent the RFI x diet interaction observed on protein turnover rates. Because on
621 average RFI groups are the same age, this BW difference implies that the pre-weaning growth
622 of the efficient RFI group was greater (estimated at around +100 g/d) compared to the
623 inefficient RFI group and this could have impacted the metabolic measurements later on.
624 Although RFI should be phenotypically independent of performance for the evaluated whole
625 population this may not be necessarily true for extreme sub-populations. Interestingly, the same
626 non-significant BW difference between extreme RFI animals fed a grass silage diet (+7%) was

observed at the end of the RFI test in our previous study (Jorge-Smeding et al., 2021) and deserves further studies to understand the origin and implications.

A trend for efficient RFI cattle to consume less oxygen by unit of BW was found regardless of the diet (Supplementary Table S2) and is consistent with the lower oxygen consumption expected as intake decreases (Blaxter, 1962). Lower oxygen consumption in efficient RFI cattle has been observed in the few studies conducted in beef cattle (Nkrumah et al., 2006; Chaves et al., 2015) and is indicative of lower energy expenditure of these animals. When the oxygen consumption (g/d) measured in the present study from the 100 animals was included in the RFI model it became a significant determinant, and slightly explained DMI variations beyond the metabolic BW and ADG effects, contributing to around 8% of the RFI variations (r2 of the RFI model changed from 0.844 to 0.851). These results should be interpreted together with differences observed in the dressing percentage across RFI groups, since energy expenditure of visceral tissues is much higher than that of the skeletal-muscle (Ortigues and Doreau, 1995). In ruminants the increment in energy expenditure with intake originates for 80% from the portal-drained viscera and liver but for only resting 5% from skeletal-muscles Ortigues-Marty et al. (2017). In the current study, we calculated energy expenditure from body composition determined at slaughter and average in vivo tissue metabolic rates (2.4 kJ/d/g wet tissue for the portal-drained viscera, 0.75 kJ/d/g wet tissue for the liver and 0.12 kJ/d/g wet tissue for the whole-carcass, Ortigues and Doreau, 1995; assuming similar metabolic rates across diets and RFI groups). The calculated whole-energy expenditure averaged 146±41 kJ/d/kg empty BW, and was similar across extreme RFI animals and diets. These results differ from trends measured with oxygen consumption, suggesting that the different energy expenditure observed between RFI extreme animals are partially but not totally explained by differences in body composition.

Protein turnover has a high energy cost (Lobley, 2003) with protein synthesis accounting for around 23% of the whole-body energy expenditure in ruminants (Caton et al., 2000). We only observed differences in the protein turnover rate of skeletal muscle of animals fed the corn silage-based diet with no significant changes at the whole-body levels for none of the two studied diets. Since differences in energy expenditure across RFI groups were similar regardless of the diet, our data suggests that changes in the skeletal muscle protein turnover rate associated with RFI in the corn silage-fed animals may have had a minor impact on energy expenditure.

Conclusions

Our results highlight that some mechanisms associated with RFI were similar across two contrasted diets, such as the NUE improvement, higher dressing percentage or lower oxygen consumption in efficient vs non-efficient RFI animals. However, our results also pointed out that differences in protein turnover in carcass muscle between RFI animals were diet-dependent. The nature of absorbed nutrients and the resulting insulin response with corn silage- vs grass silage-based diets may explain the observed RFI by diet interaction in protein turnover rates. Further studies are warranted to confirm diet-specific RFI determinants in beef cattle.

Ethics approval

The protocol of this study was approved by the Ethics Committee of the Auvergne-Rhône-Alpes region and the French Ministry of Higher Education, Research and Innovation (Authorization number: APAFIS #16194-2016101016361277 v6 delivered on 14th January 2019). This experiment was conducted at INRAE, Centre Auvergne-Rhône-Alpes, France.

Data and model availability statement

None of the data were deposited in an official repository. Data are confidential but available to reviewers upon request.

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Declaration of interest

None

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865 Xie, Y., Wu, Z., Wang, D., Liu, J., 2019. Nitrogen partitioning and microbial protein synthesis
866 in lactating dairy cows with different phenotypic residual feed intake. Journal of Animal
867 Science and Biotechnology 10, 54.

868 Table 1: Ingredients, chemical composition and feed values of the experimental diets tested
869 on fattening Charolais bulls[1]

Item	Grass silage-based diet	Corn silage-based diet
Ingredient composition (% of DM)		
Forages		
Corn silage	-	62.1 ± 0.84
Grass silage	59.2 ± 3.15	-
Wheat straw	5.08 ± 0.82	4.08 ± 0.16
Concentrate		
Wheat grain	7.68 ± 0.22	20.8 ± 0.42
Beet pulp	23.7 ± 3.22	-
Soybean meal	3.93 ± 1.47	11.5 ± 0.23
Bicarbonate	-	0.67 ± 0.01
Minerals and vitamin mix*	0.38 ± 0.01	0.35 ± 0.01
Chemical composition (g/kg of DM)		
OM	883 ± 5.75	955 ± 1.56
CP	141 ± 4.94	140 ± 8.48
NDF	454 ± 37.5	331 ± 1.41
ADF	254 ± 21.2	158 ± 10.6
Starch	48.5 ± 4.90	329 ± 1.41
Starch/NDF (g/g)	0.11 ± 0.02	0.99 ± 0.01
In vitro enzymatic digestibility[2] (%)		
DM	73.3 ± 3.01	75.3 ± 1.04
OM	74.2 ± 4.03	77.4 ± 1.02
Feed Values[3]		
Net Energy (Mcal/kg)	1.51 ± 0.09	1.66 ± 0.01
Rumen CP degradability (%)[4]	74.8 ± 1.13	72.8 ± 0.21
MP (g/kg of DM)	82.0 ± 0.00	84.9 ± 1.41
MP/Net energy (g/Mcal)[5]	54.3 ± 3.57	51.1 ± 0.46
RPB (g/kg of DM)[6]	9.00 ± 4.24	4.00 ± 5.65
Energy density (Mcal/UEB)	1.61 ± 0.13	1.87 ± 0.04

870 Abbreviations: OM = organic matter; MP = metabolizable protein; UEB = fill units for bovines
871 (INRA feeding system)
872 [1]Means and SD of experimental diets from the two years (2019 and 2020).
873 *Minerals and vitamin mix: 5 % P, 25 % Ca, 8 % Mg, 0.2 % Na, vitamin A (210 mg/kg), vitamin
874 D3 (3.75 mg/kg), vitamin E (2730 mg/kg) and vitamin B1 (4.5 mg/kg).
875 [2]Estimated from enzymatic digestion with pepsin-cellulase (Aufrère, 1982)
876 [3]Feed values were estimated from chemical composition and proportion of ingredients by
877 considering digestive interactions (INRA, 2018).
878 [4]Estimated from an enzymatic hydrolysis assay for each feed in the concentrate (Aufrère and
879 Cartailler, 1988) or from chemical composition (CP and NDF) for forages (INRA, 2018).
880 [5]Recommended values are between 53 and 48 for BW of 400 and 700 kg, respectively, in fattening
881 Charolais bulls (INRA, 2018).
882 [6]RPB = rumen protein balance, representing nitrogen intake minus non-ammonia nitrogen at the
883 duodenum (INRA, 2018).

884 Table 2: Animal performance during a 84-d feed efficiency test (Test A) in extreme residual feed intake (RFI) Charolais
885 young bulls fed either a grass-silage or corn-silage diet

Item	Grass silage-based diet		Corn silage-based diet			*P*-value		
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	RFI	Diet x RFI
Number of animals	8	8	8	8				
Animal performances								
Initial BW (kg)	440	412	429	429	16.3	0.86	0.41	0.40
Initial age (days)	294	293	294	294	12.9	0.67	0.48	0.38
Final BW (kg)	540	512	560	561	9.8	0.07	0.47	0.43
DM intake (kg/d)	7.93	8.47	8.65	9.75	0.147	0.001	0.004	0.29
Average daily gain (kg/d)‡	1.15	1.12	1.57	1.59	0.011	0.001	0.92	0.75
Feed conversion efficiency (kg/kg)	0.145	0.132	0.181	0.163	0.0036	0.001	0.04	0.68
Residual feed intake (kg/d)	-0.45	0.49	-0.58	0.48	0.012	0.58	<0.001	0.28
Adipocyte size*								
Day 0 (µm)	70.8	68.3	62.3	67.9	14.7	0.19	0.63	0.22
Day 84 (µm)	87.2	82.3	85.4	92.1	10.7	0.30	0.81	0.14
Gain (µm/d)	0.19	0.17	0.32	0.29	0.13	0.009	0.56	0.93
Ultrasound fat depth†								
Day 0 (cm)	0.26	0.23	0.25	0.25	0.016	0.63	0.26	0.27
Day 84 (cm)	0.34	0.33	0.36	0.39	0.018	0.04	0.52	0.35
Gain (µm/d)	9.52	11.9	13.1	16.6	1.70	0.09	0.09	0.96

886 ‡ Average daily gain has been calculated as the linear regression of BW over time during Test A (0-84d).
887 * Adipocytes were sampled by subcutaneous biopsy conducted in the middle of a triangle formed by the last lumbar vertebrae, tail
888 base and ischial tuberosity and measured with optic microscopy (Garcia and Agabriel, 2008).
889 † Ultrasound fat depth represents the average from four anatomical regions (back, rib, gluteus and lumbar region) measured by
890 ultrasound echography.

Table 3: Nitrogen balance of extreme residual feed intake (RFI) Charolais young bulls fed either a grass-silage or corn-silage diet

Item	Grass silage-based diet		Corn silage-based diet			*P*-value		
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	RFI	Diet x RFI
Number of animals	8	8	8	8				
BW before N balance (kg)*	576	550	599	598	15.2	0.02	0.37	0.40
Nitrogen balance								
g/kg BW/d								
N intake	0.314	0.332	0.318	0.362	0.0074	0.02	0.001	0.10
N feces	0.113	0.120	0.103	0.122	0.0039	0.33	0.002	0.15
N urine	0.121	0.124	0.123	0.143	0.0046	0.03	0.01	0.06
N retained	0.079	0.087	0.092	0.096	0.0088	0.16	0.45	0.82
N partition								
g/g N intake								
N feces	0.360	0.363	0.323	0.337	0.0092	0.001	0.33	0.51
N urine	0.388	0.381	0.387	0.397	0.0194	0.69	0.94	0.65
N use efficiency[1]	0.251	0.256	0.289	0.265	0.0212	0.26	0.65	0.49

*Nitrogen balance trials were conducted during Test B, 15 days (first group) and 32 days (second group) after the end of the first feed efficiency test (Test A)

[1]calculated as retained N by unit of N intake

Table 4: Plasma and urinary metabolites as well as urinary parameters in extreme residual feed intake (RFI) Charolais young bulls fed either a grass-silage or corn-silage diet sampled during the nitrogen balance trial (Test B)

Item	Grass silage-based diet		Corn silage-based diet			*P*-value		
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	RFI	Diet x RFI
Number of animals	8	8	8	8				
Plasma metabolites								
Insulin, µg/L	0.34	0.36	0.69	0.92	0.051	0.001	0.06	0.08
Urea, g/L	0.132	0.125	0.163	0.175	0.01	0.003	0.82	0.47
Urinary excretion[1]								
Urea, g/d	62.1	62.3	77.2	101	16.9	0.001	0.04	0.06
Creatinine, mmol/d	194	178	183	184	3.8	0.64	0.21	0.16
3-methyl-histidine, mmol/d	2680	2494	2884	3144	76.7	0.01	0.81	0.12
3-methyl-histidine /Creatinine	13.8	13.9	15.7	17.1	0.26	0.001	0.16	0.19
Urinary parameters								
Urea N/total N (g/g)	0.41	0.42	0.48	0.54	0.02	0.001	0.03	0.14

[1]Urine metabolites were analyzed from a representative sample of 24h urine total collection performed during 10d.

899 Table 5: Isotopic N kinetics in plasma and urine, and estimated fractional degradation rate of skeletal muscle proteins of
900 extreme residual feed intake (RFI) Charolais young bulls fed either a grass-silage or corn-silage diet

Item	Grass silage-based diet		Corn silage-based diet			*P*-value		
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	RFI	Diet x RFI
Number of animals[†]	8	8	8	8				
Plasma								
Initial ^{15}N enrichment ($\delta^{15}N_0$, ‰)	21.1	21.7	17.9	18.5	1.76	0.03	0.58	0.99
Final ^{15}N enrichment ($\delta^{15}N_{\square}$, ‰)	8.19	7.90	6.61	7.14	0.314	<0.001	0.60	0.08
Fractional depletion rate (k, %/d)[α]	4.38[c]	4.33[c]	4.61[b]	5.12[a]	0.001	0.001	0.11	0.04
Urine								
Initial ^{15}N enrichment ($\delta^{15}N_0$, ‰)								
Fast turnover pool	49.1	46.1	43.6	48.3	4.79	0.56	0.99	0.50
Slow turnover pool	14.8	15.4	11.8	13.9	1.28	0.01	0.37	0.76
Fractional depletion rate (k, %/d)								
Fast turnover pool	81.7	79.5	86.8	87.1	4.38	0.12	0.92	0.68
Slow turnover pool[β]	5.28	5.32	6.26	7.11	0.053	0.01	0.34	0.38
Skeletal-muscle								
FDR (%/day)[*]	1.43[b]	1.39[b]	1.40[b]	1.64[a]	0.015	0.07	0.11	0.02

901 [a-c] Averages with different letters within the same row are significantly different ($P<0.05$)
902 [†] 3 animals were removed from the urinary ^{15}N kinetics (2 individuals RFI- and 1 individual RFI+, both in the corn-based diet) because of
903 their poor bi-exponential model's fitting.
904 [α] Plasma fractional depletion rate of ^{15}N represents the fractional synthesis rate (FSR) of plasma proteins (Cantalapiedra-Hijar et al.,
905 2019).
906 [β] Urinary fractional depletion rate of ^{15}N in the slow turnover pool represents the fractional degradation rate (FDR) of the whole-body
907 protein mass (Cantalapiedra-Hijar et al., 2019).
908 [*]FDR = Fractional degradation rate estimated according to Castro-Bulle et al. (2007) from the measured urinary 3-methyl-histidine
909 excretion during N balance (Table 4) and the estimated muscle in carcass (Table 6).

910 Table 6: Carcass performances, tissue composition of the 6th rib and whole carcass, and total body chemical composition and gains
911 in extreme residual feed intake (RFI) young Charolais fed either a grass-silage or corn-silage diet

Item	Grass silage-based diet		Corn silage-based diet			*P*-values		
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	RFI	Diet x RFI
Number of animals	8	8	8	8				
Carcass weight (kg)	419	399	447	430	9.82	0.009	0.03	0.86
Dressing percentage (%)	62.0	60.7	64.4	62.0	0.42	0.003	0.003	0.18
Measured body composition								
6th rib tissue composition (%)								
Muscle	71.6	70.7	75.5	71.8	0.93	0.01	0.02	0.13
Fat	9.70	9.74	8.68	10.7	0.66	0.97	0.12	0.14
Bone	14.6	15.0	11.7	14.0	0.56	0.001	0.02	0.11
Nerves and cartilages	3.99	4.34	3.9	3.32	0.251	0.03	0.66	0.07
Muscle to Fat ratio	7.38	7.26	8.69	6.71	0.62	0.40	0.07	0.08
Estimated carcass composition								
Carcass tissue composition, %								
Muscle	70.3	70.2	71.8	70.3	0.43	0.11	0.05	0.08
Fat	14.6	14.5	14.1	15.3	0.47	0.62	0.16	0.09
Skeleton	15.1	15.3	14.1	14.4	0.24	0.87	0.12	0.61
Chemical whole-body composition, %								
Protein	19.7	19.7	19.7	19.5	0.11	0.55	0.23	0.16
Fat	14.2	13.9	14.4	15.7	0.58	0.24	0.46	0.17
Water	61.7	62.1	61.8	61.0	0.40	0.18	0.52	0.18
Mineral	4.28	4.31	4.28	4.23	0.321	0.25	0.70	0.25
Estimated body gains[1] (kg)								
Empty BW	241	227	264	261	34.3	0.02	0.47	0.62
Total protein	48.3	45.7	53.4	50.1	7.61	0.04	0.26	0.95
Total fat	45.9	40.7	47.9	54.7	15.7	0.07	0.84	0.19
Estimated nitrogen balance (0-196 d)								
N intake (g/d)	187	196	193	209	4.75	0.05	0.01	0.43

N retained (g/d)[2]	39.1	37.0	43.3	41.6	2.09	0.05	0.37	0.92
N use efficiency (g/g)	0.208	0.189	0.224	0.200	0.0125	0.29	0.08	0.83

[1]Gains were calculated as the difference between the end and the start of the experiment. Composition at the start was estimated from the diameter of subcutaneous adipose cells sampled by biopsy at the start of the experiment test and expressed as% of empty BW (Garcia and Agabriel, 2008), whereas composition at the end was estimated from the 6th rib dissection (See calculations in supplementary materials) and expressed as% of empty BW.

[2]N retained was calculated as the estimated protein gain divided by the length from the start to the end of the experimental period (196d).