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ABSTRACT

Several studies tried to identify digestive determinants of individual variation in feed efficiency between fattening bulls, because of their importance for breeding and management strategies. Most studies focused on single traits or single diet. Little is known about diet-dependent differences in digestive determinants and on their relative importance in distinguishing divergent residual feed intake (**RFI**) bulls. This research aimed (i) to identify digestive traits that differed between bulls diverging in RFI and fed a maize silage- or a grass silage-based diets; (ii) to highlight the relationships between RFI and digestive traits, and (iii) to explore the hierarchy among digestive traits in discriminating RFI divergent bulls. After an initial RFI test of 84 days on 100 Charolais growing bulls fed two different diets based on grass silage (**GS**), or maize silage (**MS**), the 32 most RFI divergent bulls were selected (eight efficient RFI− and eight inefficient RFI+ bulls per diet) and measured thereafter for total tract apparent digestibility and transit rate, enteric gas emissions (CH_4 and H_2), rumen pH, and feeding behaviour. Rumen particle size and visceral organ and reticulo-omasal orifice (**ROO**) sizes and rumen and ileum histology were measured at slaughter on the 32 selected extreme RFI bulls. Irrespective of the diet, efficient bulls (RFI−) had lower rumen size, CH_4 yield (g/kg DM intake; tendency), lower number of cells in the ileal crypts, tended to have longer time of rumen pH below 5.8 and lower proportion of small size particles in rumen content than non-efficient bulls (RFI +). A long-term test for feed efficiency (197 d on average) was performed on the whole experimental period until slaughter for the 100 animals. The long-term RFI value was negatively related to time spent in activity other than ingestion, rumination, and resting, and positively related (tendency) to the duration of ingestion events, to rumen and abomasum size, irrespective of the diet. Diet-dependent effects were noted: with GS, efficient (RFI−) bulls showed a slower transit rate, whereas with MS, efficient (RFI−) bulls tended to have shorter resting events and a smaller ROO than inefficient bulls (RFI+). The transit rate and the ROO size tended to be positively related, while total tract apparent digestibility of nitrogen was negatively related to long-term RFI value, but only in GS. Rumen size appeared as the most discriminating digestive variable between RFI divergent bulls, but this result should be validated on a larger number of animals and diets.

Implications

Differences in feed efficiency between bulls exist and can be diet-dependent. We aimed to identify the digestive determinants of feed efficiency for growing bulls fed either a grass silage or a maize silage-based diets. Irrespective of the diet, we quantified the relationships between residual feed intake and several *in vivo* and *postmortem* digestive traits. However, some relationships are diet-dependent. Irrespective of the diet, rumen size was the most discriminating variable between residual feed intake divergent bulls. Small rumen size also implies low metabolic requirement for maintenance, suggesting to pay attention to metabolic traits for future precision feeding and breeding strategies.

Introduction

Growing world demand for animal products contrasts with an increasing limitation in feed resources because of climatic change. This makes improvement in feed efficiency one of the most

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important targets in future of livestock farming systems (MacLeod et al., 2018). This is particularly relevant for beef cattle, as their feed conversion rate into meat at present is low when compared with monogastric species (30 vs 133 kg DM of feed per kg of produced protein, respectively; Mottet et al., 2017).

Management strategies at the herd level can improve overall feed efficiency, but there is also variation in feed efficiency between individual animals. Among the different parameters used to estimate individual feed efficiency, residual feed intake (**RFI**) is emerging as the preferred parameter for genetic selection, as it is not correlated with BW and performance (Cantalapiedra-Hijar et al., 2018). Several studies in recent decades have tried to identify the digestive and metabolic determinants of such differences between beef cattle (reviewed by Cantalapiedra-Hijar et al., 2018; Kenny et al., 2018) because of their importance for breeding and management (precision feeding) strategies. Most studies have focused on single traits like total tract apparent digestibility, enteric CH_4 emission, feeding behaviour (Durunna et al., 2011; Herd et al., 2016; De la Torre et al., 2019), or metabolisable energy partitioning (Nkrumah et al., 2006) and have been carried out with animals fed energy-dense diets (mostly based on maize silage). However, grass-based diets are gaining attention for fattening bulls as they have a lower carbon footprint, reduce feed-food competition, and increase meat quality (Cabiddu et al., 2022). Furthermore, recent research has highlighted that individual RFI differences could be diet-dependent (Lahart et al., 2020; Jorge-Smeding et al., 2021). When comparing the RFI of the same beef cattle fed high concentrate, grass silage and concentrate, or pasture-based diets, Lahart et al. (2020) found a weak or non-significant correlation between RFI values. Concerning metabolic determinants, Jorge-Smeding et al. (2021) observed greater adipose and plasma concentrations of branched chain amino acids and lower insulin sensitivity in high RFI bulls when fed a maize silage-based diet instead of a grass silage-based diet. Guarnido-Lopez et al. (2022) observed a lower protein turnover rate in low RFI bulls fed maize silage compared to low RFI bulls fed grass silage. Little is known about diet-dependent differences between divergent RFI bulls in digestive determinants. Durunna et al. (2011) and Bes et al. (2022) did not find any significant interaction between RFI and diet for feeding behaviour traits or enteric gas emission. We hypothesise that the digestive traits of bulls fed diets differing in fibre and starch content would affect differently their RFI.

It is known that RFI is a multitrait phenotype (Cantalapiedra-Hijar et al., 2018, Kenny et al., 2018) suggesting that several digestive traits may contribute to explain differences between divergent RFI bulls, with some traits possibly being more important than others. Studies highlighting the relationship between multiple digestive traits and RFI with contrasted diets are still missing. As a consequence, to the best of our knowledge, no studies have tried to rank the relative importance of the various digestive traits in their ability in distinguishing divergent RFI bulls.

Therefore, the aims of our research were (i) to identify digestive traits in bulls that phenotypically diverged in RFI and were fed a maize silage- or a grass silage-based diet and to highlight their diet-dependent or -independent relationships with RFI, and (ii) to explore the hierarchy among digestive traits in their ability to discriminate RFI divergent bulls.

Material and methods

Animals, diets and experimental design

The present study was carried out at the experimental farm of Herbipôle (INRAE, Theix, France, https://doi.org/10.15454/1.5572318050509348E12) during two consecutive years (October 2018–May 2019 and October 2019–March 2020). The details of the experimental design are described by Guarnido-Lopez et al. (2022). Briefly, two independent batches (one per year) each of 50 9-month-old Charolais growing bulls (382 ± 41 kg BW and 259 ± 42 days old at the beginning of the experiment) were split into two equivalent groups balanced for sire and BW at arrival, receiving individually ad libitum one of two different diets: a total mixed ration based on grass silage (59% of dietary DM) and high-fibre concentrate (**GS**), or a total mixed ration based on maize silage (62% of dietary DM) and high-starch concentrate (**MS**).

After 4 weeks of adaptation to the experimental diets, bulls (414 ± 54 kg BW and 290 ± 42 days old) were evaluated during a first test for feed efficiency (days 1–85) and classified as efficient (RFI−) or inefficient (RFI+) according to the results of the test. After this first RFI test, all the bulls were still stalled together until slaughter (day 197), except for two weeks during which the 32 most RFI divergent bulls identified by the first RFI test (four RFI− and four RFI+ per diet per batch) were housed individually in digestibility stalls for measurement of total tract apparent digestibility and transit rate. Enteric gas emission and feeding behaviour were measured throughout the experiment until slaughter (days 1–197), except during the digestibility stall-periods. Rumen pH was measured after the digestibility measurement until slaughter (days 86–197). Furthermore, rumen particle size, visceral organ size, and histology were determined at slaughter (day 197). The bulls averaged 698 ± 63 kg BW and were 485 ± 42 days old at slaughter. In parallel, feed efficiency over the whole experiment (from day 1 to slaughter) was measured and expressed as RFI among the initial population of 100 animals (25 per diet and per batch). The re-classification of the 32 studied bulls as efficient (RFI−) or inefficient (RFI+) according to the results of the long-term RFI test was also recorded.

Sampling, measurement and analysis

Intake, diet and performance

On the 100 bulls, feed intake was recorded daily for each animal using automatic weighing troughs (BioControl AS, Rakkestad, Norway). The DM intake (**DMI**) was calculated using the DM of the intake and the refusal, analysed five times (monday to friday) per week, by dehydrating samples at 103 °C for 24 h. All feed and refusal samples were analysed for organic matter (60 °C for 72 h), NDF, ADF (Van Soest et al., 1991), CP (method 968.06; AOAC, 2005) and starch (Faisant et al., 1995) contents. The BW was measured every two weeks, and average daily weight gain (**ADG**) was calculated as the linear regression of weight over time, as detailed by Guarnido-Lopez et al. (2022).

Enteric gas measurements

The detailed procedure for the measurement of enteric gas emission used in the present experiment on the 100 bulls was reported in detail by Bes et al. (2022). Briefly, enteric gas emissions were measured by use of one GreenFeed system (GreenFeed® system, C-Lock Inc., Rapid City, SD, USA) per diet (25 bulls per GF per year). In the present paper, only digestive gas (CH_4 and H_2) emissions are given. Oxygen consumption and CO_2 emission are reported by Guarnido-Lopez et al. (2022) and by Bes et al. (2022), respectively. The daily visit rate of animals to the GreenFeed and the daily pattern of gas emissions were also reported in Bes et al. (2022). Gas data were expressed as gas production (g/day) and yield (g/kg DMI).

Total tract apparent digestibility and transit rate indicator

During the digestibility tests, total faeces and urine were individually collected for ten consecutive days as described in Guarnido-Lopez et al. (2022). Briefly, total 24-h 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. Urine samples were collected daily, and at the end of the collection period, samples were homogeneously pooled per animal (1% of the total daily excretion). Total 24-h faecal excretion from each animal was collected. After weighing and mixing, two individual fresh daily aliquots of faeces (0.5% of daily excretion, each) were used for DM determination and pooled weekly before drying (60 °C, 48 h) and grinding (1-mm screen, ZM 200 Retsch Mill) for chemical analyses (organic matter, NDF, ADF, CP, starch), performed as described for feeds. Total transit rate was estimated using titanium dioxide (**TiO_2**) as a marker known to be fully recovered in faeces (Hafez et al., 1988; Titgemeyer et al., 2001; Glindemann et al., 2009). The TiO_2 was mixed with the concentrate and administered to the animal just before the total mixed ration distribution for ten consecutive days, at a rate of 12 g/day. Faeces samples were collected starting from the last day of TiO_2 administration at 0, 7, 8, 13, 14, 15, 16, 23, 24, 29, 31, 32, 34, 39, 47, 48, 55, 56, 63, 71, 72, 80, 95, 96, 103, 104, 120, 127, 128 and 135 h after the last administration and analysed for the TiO_2 content. The concentration of TiO_2 in faeces was determined after digestion with sulfuric acid and hydrogen peroxide followed by absorbance measured spectrophotometrically. This method was modified from the method of Myers et al. (2004) by adding 15 mL of 30% hydrogen peroxide instead of 10 mL and adding additional five drops before the absorbance was measured.

Kinetics of reticulo-rumen parameters

Reticulo-rumen pH was monitored on the 32 RFI extreme bulls continuously throughout the experiment using a wireless sensor (eCow, Exeter, UK). Each reticulo-rumen sensor was set up to record mean pH over 15 min. The reticulo-rumen sensor was orally administered to the animals at the end of the first efficiency test. The pH kinetics until slaughter were analysed, and daily relative reticulo-rumen pH indicators (**NpH**) were calculated following the procedure detailed by Villot et al. (2018) by filtering and normalising raw data to remove sensor drift and sensor noise. The average time per day where pH was below 6.0, 5.8 or 5.6, and the average mean, minimum and maximum daily pH, as well as the average daily NpH time below 0.3 and 0.5, NpH range, and NpH SD were calculated.

Animal feeding behaviour

At the beginning of the experiment, the 100 bulls were equipped with accelerometer collars Axel Medria®, attached to the neck, in order to record the main activity patterns (ingestion, rumination, resting, or other activity (**OA**)) and the posture (standing or lying down) of the individual at 5-minute time intervals. The technical details of the accelerometers are given in Crémilleux et al. (2022). An activity event (i.e. ingesting or rumination) was defined as a sequence of at least two consecutive bouts of the same activity (i.e. ingesting or rumination) not separated by at least 5 min of a different activity (i.e. non-ingesting or OA). The daily number (n/day) of events, the total time spent (min/day), and the average duration of an event (min/event) for each activity were calculated and were also expressed per 100 kg of BW (Bouchon et al., 2022).

Characteristics of the digestive tract at slaughtering

The 32 RFI divergent bulls were slaughtered at the INRAE experimental slaughterhouse of UE1414 Herbipôle Unit, at a rate of four animals per week (one RFI− and one RFI− per diet, slaughtered the same day) as soon as the first bull reached approximately 720 kg of BW, as detailed by Guarnido-Lopez et al. (2022).

Rumen content and visceral organ size. After slaughtering, the total rumen content was withdrawn and manually mixed, then a representative sample was collected, dried (103 °C 24 h) and sieved (2.0, 1.6, 1.0, 0.8, 0.5, 0.25, 0.1, 0.05, and <0.05 mm), according to Waldo et al. (1971). The proportion of each sieve size and the average particle size were then calculated on a DM basis. Visceral organ weights were measured as described by Guarnido-Lopez et al. (2022). The ROO diameter was measured using a calliper in three repetitions, averaged to obtain one value for each individual. The ROO surface was measured by image analysis using a photograph taken under standardised distance and lighting conditions incorporating a graduated ruler.

Rumen and ileum histology. A 2 cm × 2 cm sample of tissue was taken from the ventral rumen (about 10 cm from the ventral pillar) and the ileum (10 cm before the ileocecal valve). Samples from each bull were immersed in 4% buffered formaldehyde for 5 d, after being fixed with pins on cork discs to prevent shrinkage. Then, the rumen tissue samples were rinsed with water and photographed at 0.8× magnification using a light stereomicroscope (Nikon SMZ 1000, Tokyo, Japan) equipped with a digital camera (Nikon model DS-5M) and image analysis software (NIS – Elements AR 3.1, Nikon Tokyo, Japan) to evaluate the density of papillae (number of papillae/cm^2 mucosa). Papillae length (distance between the base and the tip of the papillae) and width (at the middle of the papillae), papillae absorbent area (determined as length × width × 2), and total absorbent surface of papillae per cm^2 mucosa (determined as length × width × 2, multiplied by papillae density) were measured for 20 randomly selected papillae for each animal.

The fixed ileum samples were rinsed with water and dehydrated in a graded ethanol series, clarified in xylene and paraffin embedded. Sections 5 µm thick were cut with a rotary microtome (Slee Cut 6062, Slee Medical, Mainz, Germany). The sections were stained with haematoxylin and eosin (H&E). Histological slides were acquired as whole slide images (WSI) by digital slide scanner (Nanozoomer S-60, Hamamatsu, Japan) for histomorphometric analysis. For each bull, 30 randomly selected crypts were measured, in a cross-section of the ileum. The digital image processing software NDP view 2.6.13 (Hamamatsu, Japan) was used to calculate the area enclosed by a manually traced outline of the crypt and of the crypt lumen, and the corresponding perimeter. No attempt to correct possible over- or under-estimation was made during the image processing, so care was taken to take measurements in identical conditions by a single operator who was blinded as to which feed efficiency group the samples belonged to. In addition to the crypt area, crypt perimeter, and crypt lumen area, the number of visible nuclei, i.e. the number of cells present on each transversal image of the crypt, was counted. The mean cell size was then determined by subtracting the crypt lumen area from the total crypt area and then dividing this value by the number of nuclei.

Feed efficiency calculations

The 32 RFI divergent within 100 bulls were attributed to the two RFI groups (RFI+ and RFI−) based on the initial feed efficiency test (from day 1 to day 85). For each batch, this initial RFI was calculated as the difference between observed DMI and expected DMI, i.e. the residual of the regression equation of observed DMI to ADG and mean metabolic BW (metabolic BW = $BW^{0.75}$) within diet, as follows:

$$\text{Observed DMI} = \beta_0 + D + \beta_1(\text{metabolic BW}) + \beta_2(\text{ADG}) + e$$

where β_0 is the intercept, D is the diet effect, β_1 is the regression coefficient for metabolic BW, β_2 is the regression coefficient for ADG, and e is the residual of the model equivalent to RFI. The distri-

bution of bulls when comparing theoretical vs observed DMI are given in Supplementary Fig. S1a and S1b for grass silage and maize silage, respectively.

The long-term RFI value was calculated a posteriori based on the whole experiment (from day 1 to slaughter), to take possible variations in RFI during the full experimental period into account. It was calculated as the difference between observed DMI and expected DMI, and corrected by the effect of the batch and of the diet within the batch as follows:

$$\text{Observed DMI} = \beta_0 + \text{CG} + \beta_1(\text{metabolic BW}) + \beta_2(\text{ADG}) + e$$

where CG (n = 4) is the effect of contemporary group, defined as the combination of the batch and the diet.

Statistical analysis

Data were analysed using SAS (version 9.4; SAS Institute, 2009). The equations of the applied model are given in Supplementary Material S1. The animal was used as a statistical unit for all the statistical analysis.

Indicator of transit rate

The transit rate was estimated from the disappearance time of TiO_2 in the faeces. Data were ln transformed and analysed by ANOVA using a MIXED model that included the fixed effects of the diet (n = 2), the RFI group (n = 2), the time (in hour) of faeces sampling (n = 30) and their interactions as fixed effects. The time of faeces sampling was treated as a repeated factor, using the animal as random factor. The time of faeces sampling when the faecal TiO_2 concentration started to significantly decrease from the initial value was set as cut-off point given by the model (38 h), as well as the time of faecal sampling after which no more significant differences were observed in the faecal residual TiO_2 (105 h). Data between the cut-offs were used to calculate a slope per animal using a linear regression procedure in which the sampling time was set as the regressive factor. The higher the absolute value of the negative slope (in ln $gTiO_2$/kg DM/h), the faster the digestive transit rate.

Effect of residual feed intake group

All the *in vivo* data were analysed by ANOVA using a GLM model that included the effect of RFI group (as established from the first test day 1 to 85), diet, batch and their interactions as fixed effects (RFI, diet, batch, RFI × diet, RFI × batch and diet × batch). For the *postmortem* data, the slaughter date was added as a random effect nested to the batch (four different slaughter dates per batch) in order to account for differences in BW across time. The triple interaction was also tested, but it was non-significant for any variable, so it was not included in the model. To simplify the presentation of results, only the mean values for RFI, diet and RFI × diet interaction are reported in tables. To compare means between groups for significant interactions, Tukey's posthoc test was applied. Effects were considered significant at a probability of $P < 0.05$, and as a tendency at $P < 0.10$.

Relations between residual feed intake and digestive traits

A high repeatability (0.77) of RFI across time was demonstrated by Guarnido-Lopez et al. (2022); however, some variation in RFI may occur during the bull's life. A general linear model was run on the RFI values from the long-term feed efficiency test to study their relationship with the RFI values from the first test, used as regressive factor. Diet, year and their interaction were used as fixed factors. The frequency distribution of the RFI values from the first test (days 1–85) and from the long-term test (days 1–197) (Fig. 1) highlights that RFI values moved from two separate and divergent groups of values (first test on days 1–85) to a continuous distribution (long-term test days 1–197). Thus, to evaluate relations between RFI calculated in the long-term test and digestive traits, a GLM was performed for those variables showing $P < 0.15$ in the previous GLM model for RFI effect or RFI × diet or RFI × batch interactions. The values of RFI were tested as regressive factor (covariate) and the effect of diet, batch, and the interaction diet × batch were considered as fixed effects. The interactions between RFI and diet and between RFI and batch were also included in the model.

Hierarchy of digestive traits discriminating the residual feed intake groups

In an attempt to estimate a hierarchy of digestive traits able to discriminate between RFI− and RFI+ bulls, linear discriminant analysis was performed and variables to be included in linear discriminant analysis were selected following two steps:

Step 1: to select the variables most discriminant between the RFI+ and RFI− groups determined from the first test (days 1–85) to be included in the discriminant analyses, a partial least squares discriminant analysis was applied on all the *in vivo* and *postmortem* data to obtain their variable importance in projection scores, which estimate the importance of each variable in the projection to the latent structure discrimination power.

Step 2: a correlation matrix was run on the residual of a GLM having the diet, the batch and their interaction as fixed effect for the *in vivo* traits and the slaughter date as a random effect nested to the batch for the *postmortem* data, to establish the correlations between variables. As significant correlations were found between all digestive traits belonging to same type of matrix or analysis (digestibility, rumen pH, feeding behaviour, gas measurements, organ size, rumen particle size, rumen histology and ileum histology), only one digestive trait within a matrix was kept for the linear discriminant analysis, to avoid a possible autocorrelation. The variable within each matrix with the highest variable importance in projection score showing significant difference for the RFI group or for the interaction between RFI and diet or batch in the GLM used to highlight the effect of RFI was used for the linear discriminant analysis.

For the evaluation of the discrimination capacity of linear discriminant analysis, the sensitivity (calculated as the true-positive rate) and specificity (calculated as the true-negative rate) were used, according to Fawcett (2006). Accordingly, in the current study, the sensitivity and specificity express the error rate within a group to be discriminated (RFI− or RFI+, respectively) (Fawcett, 2006). The correlation coefficients between discriminating vari-

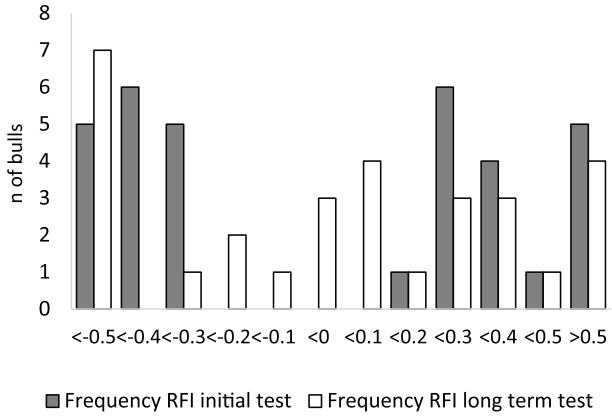

Fig. 1. Frequency distribution of the 32 bulls' residual feed intake (RFI) values determined from the initial test (days 1–85) and the long-term test (day 1–slaughter).

Table 1
Effect of residual feed intake (RFI) group, diet and their interaction on *in vivo* digestive traits of the 32 RFI divergent bulls.

Item	RFI group		Diet		GS diet[1]		MS diet[1]		SEM	*P*-value					
	RFI−	RFI+	GS	MS	RFI−	RFI+	RFI−	RFI+		RFI	Diet	Batch	RFI × Diet	RFI × Batch	Diet × Batch
Apparent total tract digestibility (%)															
DM	72.1	72.0	73.2	70.9	73.3	73.0	70.9	71.0	0.43	0.851	<0.001	0.001	0.679	0.172	0.006
OM	75.1	74.9	76.5	73.5	76.7	76.3	73.5	73.6	0.49	0.750	<0.001	0.001	0.613	0.365	0.301
NDF	61.7	62.0	73.2	50.6	73.6	72.7	49.8	51.3	1.64	0.852	<0.001	0.015	0.461	0.590	0.006
ADF	61.9	61.9	72.7	51.1	73.3	72.1	50.6	51.7	1.93	0.983	<0.001	0.809	0.565	0.757	0.248
Nitrogen	65.3	64.6	63.8	66.1	64.0	63.7	66.7	65.4	0.83	0.354	0.012	0.883	0.535	0.137	0.001
Starch	72.1	72.0	73.2	70.9	73.3	73.0	70.9	71.0	0.43	0.851	<0.001	0.001	0.679	0.172	0.006
Indicator of total tract transit rate ([Δ ln $gTiO_2$/kg faecal DM]/h)	−0.022	−0.024	−0.020	−0.026	−0.017[b]	−0.023[a]	−0.026[a]	−0.025[a]	0.0019	0.250	0.012	0.127	0.049	0.477	0.325
Enteric gas measurement															
CH_4 (g/kg DMI)	28.4	27.1	28.5	26.9	29.4	27.7	27.3	26.5	0.67	0.087	0.030	0.035	0.504	0.102	0.019
H_2 (g/kg DMI)	0.132	0.116	0.135	0.114	0.150	0.119	0.115	0.113	0.010	0.148	0.067	0.430	0.194	0.157	0.156
Ruminal pH															
Time NpH < 0.3 (min/day)	47	51	50	49	23	76	71	26	38.3	0.909	0.978	0.651	0.210	0.349	0.449
pH mean	6.26	6.35	6.32	6.29	6.31	6.34	6.21	6.37	0.067	0.172	0.609	0.157	0.345	0.132	0.441
Time at pH < 5.8 (min/day)	107	4	12	99	23	2	192	6	59.3	0.093	0.158	0.110	0.177	0.118	0.206
NpH range	0.55	0.56	0.48	0.63	0.48	0.49	0.62	0.64	0.037	0.795	0.001	0.323	0.887	0.168	0.044
NpH SD	0.11	0.11	0.09	0.14	0.09	0.09	0.14	0.14	0.01	0.890	<0.001	0.950	0.731	0.331	0.523

Abbreviations: DMI = DM intake; GS = grass silage-based diet; MS = maize silage-based diet; NpH = normalised pH; OM = organic matter; RFI = Residual feed intake; RFI+ = inefficient bulls; RFI− = efficient bulls.
[1] Values of the interaction between RFI and diet within a row with different superscripts differ significantly at $P < 0.05$.

Table 2
Effect of residual feed intake (RFI) group, diet and their interaction on feeding behaviour of the 32 RFI divergent bulls.

Item	RFI group		Diet		GS diet		MS diet		SEM	*P*-value					
	RFI−	RFI+	GS	MS	RFI−	RFI+	RFI−	RFI+		RFI	Diet	Batch	RFI × Diet	RFI × Batch	Diet × Batch
Feeding behaviour															
Ingestion time (min/day)	181	191	187	185	189	186	173	197	10.0	0.320	0.778	0.015	0.181	0.879	0.924
Ingestion events (n)	8.5	8.8	8.9	8.5	8.9	8.8	8.2	8.8	0.34	0.405	0.251	0.027	0.364	0.057	0.969
Ingestion event duration (min)	21.2	22.1	21.3	22.0	21.3	21.3	21.1	22.8	0.72	0.250	0.333	0.062	0.237	0.087	0.950
Rumination time (min/day)	450	440	466	425	466	466	435	414	12.2	0.418	0.002	0.852	0.392	0.462	0.916
Rumination events (n)	15.9	15.4	16.1	15.2	16.3	15.9	15.4	15.0	0.45	0.322	0.051	0.040	0.967	0.191	0.644
Rumination event duration (min)	28.9	29.3	29.5	28.7	28.8	30.2	29.0	28.4	1.11	0.711	0.501	0.215	0.372	0.804	0.628
Resting time (min/day)	443	464	420	487	413	427	473	501	15.9	0.198	<0.001	0.809	0.700	0.210	0.900
Resting events (n)	17.4	17.6	17.6	17.4	17.1	18.0	17.7	17.2	0.57	0.661	0.806	0.934	0.234	0.369	0.638
Resting event duration (min)	26.4	27.3	24.7	29.0	25.2	24.2	27.6	30.4	0.96	0.374	<0.001	0.825	0.060	0.037	0.778
OA time (min/day)	363	345	366	342	370	361	355	328	11.9	0.140	0.051	0.004	0.435	0.583	0.998
OA events (n)	16.8	16.9	17.0	16.7	16.9	17.1	16.7	16.6	0.30	0.807	0.276	0.732	0.656	0.102	0.637
OA event duration (min)	22.2	21.0	22.2	21.0	22.5	21.8	21.9	20.1	0.95	0.208	0.235	0.026	0.580	0.843	0.721

Abbreviations: GS = grass silage-based diet; MS = maize silage-based diet; OA = activities other than ingestion, rumination, and resting; RFI = Residual feed intake; RFI + = inefficient bulls; RFI− = efficient bulls.

Table 3
Effect of residual feed intake (RFI) group, diet and their interaction on the characteristics of digestive tract at slaughtering of the 32 RFI divergent bulls.

Item	RFI group		Diet		GS diet		MS diet		SEM	*P*-value					
	RFI−	RFI+	GS	MS	RFI−	RFI+	RFI−	RFI+		RFI	Diet	Batch	RFI × Diet	RFI × Batch	Diet × Batch
Rumen content particle size (%)															
Sieve 2.0 mm	14.52	12.17	7.67	19.02	8.94	6.40	20.09	17.95	1.708	0.182	<0.001	0.031	0.908	0.334	0.223
Sieve 1.6 mm	9.44	9.45	9.80	9.09	9.31	10.29	9.56	8.62	0.673	0.981	0.298	0.069	0.167	0.068	0.143
Sieve 1.0 mm	24.46	23.80	26.92	21.34	27.09	26.76	21.83	20.84	0.849	0.443	<0.001	0.119	0.704	0.470	0.173
Sieve 0.8 mm	9.17	9.35	9.93	8.59	9.91	9.95	8.43	8.75	0.280	0.533	<0.001	0.468	0.624	0.159	0.875
Sieve 0.5 mm	15.22	15.92	15.31	15.82	14.99	15.63	15.44	16.20	0.584	0.243	0.390	0.206	0.917	0.159	0.027
Sieve 0.25 mm	14.15	15.35	14.61	14.89	14.42	14.81	13.89	15.89	0.642	0.074	0.670	0.021	0.222	0.471	0.166
Sieve 0.1 mm	9.10	9.62	10.73	7.99	10.50	10.97	7.71	8.28	0.867	0.554	0.004	0.543	0.953	0.999	0.716
Sieve 0.05 mm	2.59	2.78	3.23	2.15	3.08	3.37	2.09	2.20	0.321	0.544	0.002	0.305	0.775	0.804	0.623
Sieve <0.05 mm	1.15	1.17	1.46	0.87	1.47	1.45	0.84	0.89	0.164	0.920	0.001	0.037	0.821	0.838	0.282
Average rumen particle size (mm)	1.18	1.11	1.02	1.26	1.05	0.99	1.30	1.22	0.050	0.195	<0.001	0.035	0.831	0.288	0.288
ROO diameter (mm)	37.6	40.5	42.8	35.2	42.9	42.7	32.2	38.2	1.97	0.139	0.026	0.269	0.062	0.242	0.017
ROO area (cm^2)	6.2	7.0	7.7	5.6	7.8	7.5	4.6	6.6	0.86	0.436	0.151	0.203	0.092	0.145	0.130
Rumen histology															
Papillae width (cm)	0.25	0.25	0.30	0.21	0.29	0.30	0.22	0.19	0.030	0.863	0.046	0.678	0.439	0.911	0.392
Papillae length (cm)	0.94	0.87	0.91	0.90	0.92	0.91	0.97	0.83	0.700	0.261	0.611	0.292	0.254	0.437	0.233
Papillae density (n/cm^2)	36.6	40.1	39.2	37.5	37.8	40.6	35.5	39.6	3.76	0.353	0.920	0.048	0.824	0.385	0.339
Papillae absorbent area (cm^2)	0.48	0.43	0.52	0.38	0.51	0.53	0.44	0.33	0.058	0.412	0.065	0.609	0.180	0.365	0.790
Absorbent surface of papillae (cm^2/cm^2 mucosa)	17.1	17.1	20.7	13.6	20.0	21.3	14.3	12.9	2.24	0.965	0.031	0.017	0.441	0.868	0.452
Ileum histology															
Crypt area (μm^2)	2 555	2 778	2 312	3 021	2 300	2 325	2 810	3 232	164.3	0.103	0.015	0.010	0.148	0.933	0.015
Crypt perimeter (μm)	184	192	177	198	177	177	191	206	5.8	0.104	0.031	0.007	0.102	0.965	0.032
Crypt lumen area (μm^2)	72.3	65.3	71.1	66.5	76.3	65.9	68.3	64.8	10.61	0.512	0.740	0.015	0.690	0.563	0.973
Number of cells in the ileal crypts (n)	25.1	27.2	25.1	27.2	24.3	25.9	25.8	28.5	1.49	0.042	0.405	<0.001	0.626	0.172	0.372
Average cell size (μm^2)	99.3	101.5	90.9	109.8	92.2	89.7	106.4	113.3	4.24	0.810	0.012	<0.001	0.181	0.158	0.014

Abbreviations: GS = grass silage-based diet; MS = maize silage-based diet; RFI = Residual feed intake; RFI+ = inefficient bulls; RFI− = efficient bulls; ROO = Reticulo-omasal orifice.

ables and the standardised canonical discriminant function of linear discriminant analysis were used to rank the importance of the variables for the discrimination between RFI− and RFI+ bulls.

Results

Effect of residual feed intake group and its interaction with diet

The results of RFI, animal performance, and visceral organ weight of the 32 RFI divergent bulls have been published by Guarnido-Lopez et al. (2022), so are not reported in the table. Briefly, irrespective of the diet, the RFI values at the first test (1–85 days) were −0.52 and 0.49 kg DM/day, for the RFI− and RFI+ groups, respectively. The growth rate of the bulls did not differ between divergent RFI groups (similar ADG: 1.36 ± 0.011 kg/day; and BW before bleeding: 543 ± 9.8 kg), regardless of a lower DMI (8.29 vs 9.11 kg DM/day, respectively; $P = 0.004$) for RFI− than RFI+ bulls. Efficient animals had higher carcass weight (443 vs 415 kg, respectively; $P = 0.03$) and lower rumen size (1.65 vs 1.80% of empty BW, respectively; $P = 0.001$) than non-efficient ones.

The effects of RFI group, diet and their interaction on the *in vivo* feeding behaviour and *postmortem* digestive traits of the 32 divergent bulls of the present experiment are given in Tables 1, 2 and 3, respectively.

Irrespective of the diet, no differences were observed between RFI divergent bulls in terms of digestibility and CH_4 production (g/day), but the CH_4 yield (g/kg DMI) tended to be higher (+4.7%; $P < 0.1$) in RFI− than in RFI+ bulls. Among rumen pH parameters, only the part of the day where pH was below 5.8 tended to be

Table 4
Relations between long-term residual feed intake (RFI) values (explanatory variable) and *in vivo* digestive traits (explained variables) of the 32 bulls, according to the diet.

Item	Intercept[1]	Batch[1]	Diet[1]	RFI[1]	RFI × Diet[1]	RFI × Batch[1]	*P*-value					
		1	GS		GS	1	RFI	Diet	Batch	RFI × Diet	RFI × Batch	Diet × Batch
Nitrogen total tract apparent digestibility (%)	67.5 ± 0.76		−5.6 ± 1.15		2.9 ± 1.59		0.539	0.003	0.620	0.081	0.329	0.001
Gas measurement												
CH_4 (g/kg DMI)	27.0 ± 0.85		0.2 ± 1.25				0.246	0.041	0.071	0.262	0.557	0.070
H_2 (g/kg DMI)	0.12 ± 0.013						0.517	0.101	0.579	0.245	0.643	0.344
Indicator of total tract transit rate ([Δ ln $gTiO_2$/kg faecal DM]/h)	−0.028 ± 0.00175		0.0085 ± 0.00262	0.0024 ± 0.00373	−0.0099 ± 0.00363		0.096	0.005	0.304	0.012	0.563	0.114
Ruminal pH												
Time at pH < 6.0 (min)		318 ± 119.8					0.719	0.204	0.004	0.100	0.287	0.690
Time at pH < 5.8 (min)		189 ± 85.7	−36 ± 90.9				0.622	0.096	0.076	0.148	0.347	0.263
Time NpH < 0.5 (min)	6.07 ± 0.063		0.04 ± 0.095				0.860	0.054	0.129	0.128	0.169	0.171
Feeding behaviour												
Ingestion events (n)	8.9 ± 0.33	−0.9 ± 0.47					0.447	0.174	0.026	0.989	0.148	0.944
Ingestion cycle duration (min)	22.6 ± 0.74			3.3 ± 1.57		−3.2 ± 1.74	0.064	0.195	0.202	0.944	0.081	0.714
Resting event duration (min)	28.7 ± 0.99		−4.3 ± 1.49			−4.6 ± 2.36	0.319	<0.001	0.726	0.124	0.063	0.856
OA time (min)	324 ± 10.7	37 ± 15.1	38 ± 16.1	−56 ± 22.9		45 ± 25.4	0.005	0.012	0.017	0.428	0.086	0.471
OA cycles (n)	16.6 ± 0.32						0.383	0.349	0.617	0.589	0.966	0.829
Ingestion cycle duration (min/100 kg BW)	3.4 ± 0.16						0.264	0.801	0.353	0.867	0.317	0.570
Rumination time (min/100 kg BW)	73 ± 3.3	9 ± 4.7	−10 ± 5.0	12 ± 7.1			0.100	0.019	0.006	0.925	0.240	0.755
Resting event duration (min/100 kg BW)	4.3 ± 0.19	0.7 ± 0.3	−0.5 ± 0.28				0.594	0.012	0.004	0.163	0.278	0.821

Abbreviations: DMI = DM intake; OA = Activities other than ingestion, rumination, and resting.
[1] Coefficients ± SE; Values are coefficients for batch 1 and grass silage-based diet (GS) if the effects are significant or trend ($P < 0.10$); coefficients are equal to zero for batch = 2 and for diet = maize silage-based diet (MS).

Table 5
Relation between long-term residual feed intake (RFI) value (explanatory variable) and *postmortem* digestive traits (explained variables) of the 32 bulls, according to the diet.

Item	Intercept[1]	Batch[1]	Diet[1]	RFI[1]	RFI × Diet[1]	RFI × Batch[1]	*P*-value				
		1	GS		GS	1	RFI	Diet	Batch	RFI × Diet	RFI × Batch	Diet × Batch
ROO diameter (mm)	40.3 ± 1.67				−6.7 ± 3.48		0.737	0.653	0.248	0.066	0.723	0.754
ROO area (cm^2)	6.1 ± 0.65				−2.7 ± 1.36		0.728	0.487	0.369	0.061	0.259	0.943
Rumen content particle size (%)												
Sieve 1.6 mm	7.9 ± 0.68	2.5 ± 0.97				−3.0 ± 1.62	0.447	0.614	0.029	0.247	0.080	0.275
Sieve 0.25 mm	16.1 ± 0.65	189.4 ± 85.72					0.221	0.755	0.032	0.716	0.636	0.135
Empty BW (kg)	644 ± 16.1	−45 ± 18.8					0.562	0.146	0.001	0.824	0.595	0.575
Visceral organ size[2]												
Full whole digestive tract (kg)	106 ± 4.6						0.208	0.632	0.008	0.575	0.285	0.210
Rumen (kg)	10.8 ± 0.37	−1.6 ± 0.47	1.1 ± 0.43				0.149	0.005	<0.0001	0.302	0.652	0.773
Rumen (% empty BW)	1.68 ± 0.051	−0.14 ± 0.072	0.22 ± 0.076	0.18 ± 0.109			0.097	<0.001	0.026	0.563	0.539	0.848
Abomasum (% empty BW)	0.33 ± 0.015			0.09 ± 0.032		−0.07 ± 0.035	0.018	0.290	0.985	0.640	0.045	0.325
Omasum (kg)	4.7 ± 0.57		1.5 ± 0.66				0.323	0.008	0.321	0.953	0.197	0.930
Omasum (% empty BW)	0.73 ± 0.082						0.378	0.002	0.828	0.837	0.211	0.878
Digestive content (kg)	74 ± 3.9	−14 ± 5.4					0.222	0.917	0.048	0.540	0.211	0.182
Digestive content (% empty BW)	11.4 ± 0.50		0.3 ± 0.10				0.175	0.412	0.540	0.558	0.166	0.079
Ileum histology												
Crypt area (μm^2)	2 883 ± 155.2	−218 ± 213.1					0.275	0.608	0.049	0.349	0.895	0.510
Crypt perimeter (μm)	196 ± 5.1	−10 ± 7.1					0.239	0.692	0.024	0.255	0.842	0.578
Number of cells in the ileal crypts (n)	31.8 ± 1.21	−8.9 ± 1.66		5.0 ± 2.45			0.034	0.887	<0.0001	0.792	0.129	0.568

Abbreviations: GS = Grass silage-based diet; RFI = Residual feed intake; ROO = Reticulo-omasal orifice.
[1] Coefficients ± SE; Values are coefficients for batch 1 and grass silage-based diet (GS) if the effects are significant or trend ($P < 0.10$); coefficients are equal to zero for batch = 2, and maize silage-based diet (MS).
[2] Data presented in the supplementary materials by Guarnido-Lopez et al. (2022).

higher for RFI− (+103 min; $P < 0.1$) compared to RFI+ bulls. The RFI− bulls rumen content tended to have a lower proportion of particles retained in the 0.25 mm sieve (−7.8%; $P < 0.1$) than those of RFI+ bulls. The number of cells in the ileal crypts was lower for RFI− than for RFI+ bulls (−7.9%; $P < 0.05$).

Diet-dependent effects were noted. The indicator of transit rate differed between divergent RFI bulls when bulls were fed the GS diet, where RFI− bulls had a 25.8% slower transit rate than RFI+ ones ($P < 0.05$), but the indicator of transit rate did not differ between RFI groups for bulls fed the MS diet. The RFI− bulls tended to have shorter resting events than RFI+ bulls (−9.1%; $P < 0.10$) on the MS diet, but no differences were found on the GS diet. The ROO area and diameter tended to be smaller in RFI− than in RFI+ bulls (−30.3% and −15.5%, respectively; $P < 0.1$) on the MS diet, but no differences were seen on the GS diet.

Relations between residual feed intake values and digestive traits

The RFI values of the 32 extreme divergent bulls from the first feed efficiency test were significantly related to those calculated from the long-term one ($P < 0.001$; $R^2 = 0.51$). Three bulls fed grass silage classified as RFI− during the first test changed their classification with the long-term test, but for two of the three bulls, RFI values were almost equal to zero. The linear relations between long-term RFI values and *in vivo* and *postmortem* digestive traits of the 32 studied bulls are reported in Tables 4 and 5, respectively. Among the tested *in vivo* and *postmortem* digestive traits, few showed a significant linear relationship with long-term RFI values. Irrespective of the diet, the time of feeding behaviour allocated to OA was negatively related to long-term RFI values (−55.7 min per 1 RFI unit; $P < 0.05$), whereas long-term RFI values tended to be positively related to the duration of ingestion events (+3.3 min per 1 RFI unit). Relative rumen size tended to be positively related and relative abomasum size was positively related to long-term RFI values (+0.18% of BW and +0.09% of BW per 1 RFI unit; $P < 0.10$ and $P < 0.05$, respectively). The number of cells in the ileal crypts was positively related to long-term RFI values (+5.0 per 1 unit of RFI; $P < 0.05$).

Concerning diet-dependent relationships, the nitrogen total tract apparent digestibility tended to be positively related to long-term RFI values only on GS (+2.9% per 1 RFI unit; $P < 0.1$). On GS diet only, the transit rate indicator was positively related to long-term RFI values (the absolute value of the TiO_2 slope increased by 0.0075 per 1 RFI unit; $P < 0.05$). Similarly, the long-term RFI values tended to be negatively related to the ROO area and the diameter on GS diet (−2.67 cm^2 and −6.69 cm per 1 RFI unit, respectively; $P < 0.1$), but the relation was not significant for MS diet.

Hierarchy of digestive traits discriminating the residual feed intake groups

The variable importance in projection score derived from the partial least squares discriminant analysis and the correlation matrix of the *in vivo* and *postmortem* digestive traits of the 32 RFI divergent bulls are given in Supplementary Table S1. The linear discriminant analysis showed a sensitivity of 83% and a specificity of 88%. Table 6 reports the correlation coefficients between discriminating digestive traits and the standardised canonical discriminant functions of linear discriminant analysis. Rumen size was the most discriminating trait. A correlation coefficient almost half that of rumen size was observed for the average time per day of rumen pH < 5.8. The ROO diameter, rumen content particle size and CH_4 yield also showed similar reciprocal correlation coefficient ranking and correlation coefficients were slightly lower than those of pH data. The number of cells in the ileal crypts, the indicator of

Table 6
Absolute correlation coefficients between discriminating digestive traits and the standardised canonical discriminant functions of the linear discriminant analysis performed to discriminate bulls divergent for residual feed intake (RFI).

Variable[1]	Absolute correlation coefficient
Rumen size (% empty BW)	0.69
Time at pH < 5.8 (min/day)	0.34
ROO diameter (mm)	0.28
Rumen particle size sieve 0.25 mm (%)	0.28
CH_4 yield (g/kg DMI)	0.24
Cells in the ileal crypts (n)	0.18
Indicator of total tract transit rate ([Δ ln g TiO_2/kg faecal DM]/h)	0.10
Resting time (min)	0.02

Abbreviation: DMI = DM intake; ROO = Reticulo-omasal orifice.
[1] The variables used were those with the highest variable importance in projection within each matrix, and showing significant differences for RFI group in Tables 1–3.

total tract transit rate, and the resting time had lower correlation coefficients than the other variables.

Discussion

Relationship between residual feed intake and digestive traits and interaction with diet

The difference in the RFI values obtained in the first feed efficiency test between the RFI+ and RFI− groups was in line with those reported by other studies comparing RFI divergent groups of animals (Castro Bulle et al., 2007; Lines et al., 2014; Kelly et al., 2014). In general, the results obtained by ANOVA with RFI groups determined according to the first feed efficiency test, were confirmed by regression using the RFI values obtained by the long-term feed efficiency test. This was despite some changes in RFI values and classification between the first to the long-term feed efficiency test. This result is particularly relevant as it confirms the high repeatability of RFI across time (Guarnido-Lopez et al., 2022).

The lighter rumen-reticulum in efficient bulls irrespective of the diet observed in our trial (Guarnido-Lopez et al., 2022) is in agreement with the results of Fitzsimons et al. (2014) and Kenny et al. (2018), with genetic studies by Taussat et al. (2019), and is consistent with the lower DMI of efficient animals. Because of their high metabolic rate, bigger digestive organs for inefficient animals may be associated with a higher metabolic energy consumption for maintenance (Basarab et al., 2003).

A surprising result of our trial was the lack of difference in total tract apparent digestibility traits between RFI divergent bulls irrespective of the diet, as a higher total tract apparent DM digestibility was observed in the literature for efficient compared to inefficient cattle (between +1.4 and +4%; Bonilha et al., 2017; De La Torre et al., 2019), probably due to a longer retention time of digesta in the rumen (Sauvant and Nozière. 2016). A slower transit rate for efficient RFI animals due to lower DMI was also hypothesised to explain their higher total tract apparent digestibility and CH_4 yield (Cantalapiedra-Hijar et al., 2018). However, RFI− bulls in our trial showed a slower transit rate only on the GS diet, whereas no interactions between the RFI group and diet were observed for total tract apparent digestibility and CH_4 yield.

The lack of differences in rumen histological traits between RFI divergent bulls, regardless of the diet, seems to exclude a possible role of absorbing ruminal surface characteristics as determinant of variability in individual RFI.

Rumination time (min/100 kg BW) and duration of ingestion (min) events were positively related to RFI, irrespective of the diet. This has also been reported by Kelly et al. (2014) and Lahart et al. (2020), who showed positive correlations between RFI and ingestion and rumination behaviour in heifers fed maize silage and in grazing cattle, respectively. Kelly et al. (2014) concluded that efficient animals may spend less time eating and ruminating, thereby utilising less energy for digestive activities. This is in agreement with the negative relationship we observed between RFI and the time spent in OA. This feeding behaviour pattern can probably be partially explained also by the positive relation we observed between RFI and rumen and abomasum size (as % of empty BW). A smaller rumen may reduce energy expenditure of visceral tissues, but also needs a shorter ingestion time to be filled, with a consequently lower rumination time.

A higher number of cells in the ileal crypts for RFI+ bulls seem to be at odds with the results of Montanholi et al. (2013). In their experiment, however, RFI− steers had greater ADG and final BW than RFI+ (Mader et al., 2008), suggesting a positive effect of growth and size coupled to efficiency. In our trial, no differences in growing performances (ADG, BW) were observed between RFI groups. A higher number of cells in the ileal crypts of RFI+ bulls could be explained by a higher mucus secretion because of a higher DMI or a higher intestinal cell turnover rate, as reported in monogastrics (Piel et al., 2005; Metzler-Zebeli et al., 2017). A higher intestinal cell turnover rate might be in agreement with the larger rumen size, indicating higher energy demands for maintenance of the gastrointestinal tract, as reported in monogastrics (Metzler-Zebeli et al., 2017).

Concerning the diet-dependent relationship between RFI and digestive traits, the slower transit rate for efficient bulls fed GS diet was expected in relation to their lower DMI, and the significance of the negative relationship between RFI and transit rate in GS diet only can be related to the higher proportion of small particle size in the rumen. Indeed, the increase in transit rate with DMI was shown to be higher for a diet rich in small particles (Sauvant and Nozière, 2016). A larger size of the ROO in non-efficient RFI+ bulls fed MS did not seem to have any effect on transit rate. This is supported by the fact that a similar ROO size was accompanied by different transit rates for divergent RFI bulls fed GS.

Hierarchy of digestive traits discriminating the residual feed intake groups

To the best of our knowledge, this is the first study to attempt to hierarchise the importance of digestive traits in discriminating between RFI divergent bulls. It is not surprising that rumen size appeared to be the most important variable, as it is known that rumen size varies with DMI (Basarab et al., 2003), even if it is not clear if rumen size is a direct consequence of intake or vice-versa. In the present experiment, rumen size was measured by organ weighing *postmortem*, but rapid *in vivo* measurements of abdominal volume by 3D imaging are being developed (Le Cozler et al., 2019) and have been successfully proposed to distinguish between efficient and inefficient cows (Cantalapiedra-Hijar et al., 2020) and thereby to be used as a proxy to discriminate RFI divergent bulls for phenotyping. Even if the other digestive traits only have a minor weight compared to rumen size in their ability to discriminate between diet-divergent bulls, their role was important, confirming that RFI is a multitrait phenotype (Cantalapiedra-Hijar et al., 2018, Kenny et al., 2018). Among the other digestive traits, rumen particle size and ROO size are *postmortem* measurements and it would be difficult to imagine their use for phenotyping at the current stage of knowledge. Measurements of rumen pH and CH_4 yield are possible *in vivo*, but are difficult and expensive to perform, so their routine use is not completely justified, especially considering their moderate weight in the discrimination.

In the present experiment, we included rumen size in the digestive traits, as we considered its involvement in the digestive process. However, visceral organs consume about 60% of theoretical

maintenance metabolic energy requirements (Ortigues-Marty et al., 2017), so greater rumen size may result in higher metabolic energy consumption for maintenance (Basarab et al., 2003). Indeed, Guarnido-Lopez et al. (2022) observed a higher O_2 consumption for RFI+ bulls associated with a greater rumen size. Thus, the high weight in discrimination of rumen size may suggest a greater importance of metabolic rather than digestive traits in discriminating between divergent RFI bulls. Further studies are needed to explore such a hypothesis. The relatively small number of animals included in the present experiment calls for care in generalising the hierarchy among the digestive traits in the discrimination between efficient and inefficient bulls. Our results should be validated in a large number of animals.

In conclusion, RFI divergent bulls differed in a few digestive traits, some of which were diet-dependent, with differences observed mainly when fed a fibre-rich grass silage-based diet. Among all digestive traits, rumen size appeared to be the most discriminating variable between RFI divergent bulls, with efficient bulls having a smaller rumen. These results should be validated on a larger number of animals and diets.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.101013.

Ethical 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 (Authorisation 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 have been deposited in an official repository. The data that support the study findings are confidential.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

None.

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