GenTORE

Genomic management Tools to Optimise Resilience and Efficiency

Grant agreement n°: 727213

H2020 - Research and Innovation Action

D2.1

Peer-reviewed paper on extension of RFI methodology to quantify the relative importance of efficiency components over time and nutritional environments

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Due date: M30

Actual submission date: M30
Resubmission date: M48

Project start date: 1st June 2017   **Duration:** 60 months

Workpackage concerned: WP2

Concerned workpackage leader: Didier Boichard (INRAE)

Lead Beneficiary: INRAE

Dissemination level:

   X **PU:** Public (must be available on the website)
   ☐ **CO:** Confidential, only for members of the consortium (including the Commission Services)
   ☐ **CI:** Classified, as referred to in Commission Decision 2001/844/EC

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1. Summary

In this deliverable new extensions of the Residual Feed Intake methodology for phenotyping feed efficiency were developed and tested in both dairy and beef cattle using a common database of observations created in WP 2 using data provided by the GenTORE partners.

2. Introduction

Residual Feed Intake methodology is widely used to quantify the differences between animals in feed efficiency and as such provides a key phenotype for use in genetic selection for efficiency. However, traditional RFI methods were not designed for use with repeated time-series measurements. Accordingly, this deliverable reports on work done to extend the RFI methodology to better deal with such measurements, which are increasingly becoming available in the context of precision livestock farming.

3. Results

3.1 Results 1

Results for dairy cow data are presented in **Annex 1**. This manuscript has been published with the following reference:
Martin P, Ducrocq V, Gordo DGM, Friggens NC. 2021. Animal, 15, 100101.
http://doi.org/10.1016/j.animal.2020.100101

3.2 Results 2

Results for beef cattle data are presented in **Annex 2**. This manuscript presents the methodology of RFI analysis in beef cattle and its application to WP2.1 database. The following published paper uses this methodology and applies it to study ruminal microbiology in this database.
Costa-Roura S., Villalba D., Blanco M., Casasús I., Balcells J., Seradj A.R. 2021. Ruminal microbiota is associated with feed-efficiency phenotype of fattening bulls fed high-concentrate diets. Animal Production Science, https://doi.org/10.1071/AN20344

4. Conclusions

Significant progress in RFI methodology has been made that will facilitate the phenotyping of this complex trait using time-series data. Further, the models developed are compatible with the requirements for extension to genetic evaluation models.

5. Partners involved in the work

INRAE, CITA, UDL (current work on methodology)
AU, CITA, DLO, IDELE, INRAE, LfL, TEAGASC, UDL, UNIPD (made data available)

Annex 1 – Results for dairy cow data

A new method to estimate residual feed intake in dairy cattle using time series data.

Annex 2 - Results for beef cattle data

Methodology approach of Residual Feed Intake calculation for beef data

A new method to estimate residual feed intake in dairy cattle using time series data

P. Martin [a,*], V. Ducrocq [a], D.G.M. Gordo [b], N.C. Friggens [c]

[a] UMR GABI, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[b] Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, DK-8830 Tjele, Denmark
[c] UMR MoSAR, INRAE, AgroParisTech, Université Paris-Saclay, 75005 Paris, France

ARTICLE INFO

ABSTRACT

In dairy, the usual way to measure feed efficiency is through the residual feed intake (**RFI**) method. However, this method is, in its classical form, a linear regression, which, by construction, does not take into account the evolution of the RFI components across time, inducing approximations in the results. We present here a new approach that incorporates the dynamic dimension of the data. Using a multitrait random regression model, the correlations between milk, live weight, DM intake (**DMI**) and body condition score (**BCS**) were investigated across the lactation. In addition, at each time point, by a matrix regression on the variance–covariance matrix and on the animal effects from the three predictor traits, a predicted animal effect for intake was estimated, which, by difference with the actual animal effect for intake, gave a RFI estimation. This model was tested on historical data from the Aarhus University experimental farm (1 469 lactations out of 740 cows). Correlations between animal effects were positive and high for milk and DMI and for weight and DMI, with a maximum mid-lactation, stable across time at around 0.4 for weight and BCS, and slowly decreasing along the lactation for milk and weight, DMI and BCS, and milk and BCS. At the Legendre polynomial coefficient scale, the correlations were estimated with a high accuracy (averaged SE of 0.04, min = 0.02, max = 0.05). The predicted animal effect for intake was always extremely highly correlated with the milk production and highly correlated with BW for the most part of the lactation, but only slightly correlated with BCS, with the correlation becoming negative in the second half of the lactation. The estimated RFI possessed all the characteristics of a classical RFI, with a mean at zero at each time point and a phenotypic independence from its predictors. The correlation between the averaged RFI over the lactation and RFI at each time point was always positive and above 0.5, and maximum mid-lactation (>0.9). The model performed reasonably well in the presence of missing data. This approach allows a dynamic estimation of the traits, free from all time-related issues inherent to the traditional RFI methodology, and can easily be adapted and used in a genetic or genomic selection context.

Implications

This paper proposes a new methodology to estimate feed efficiency in a continuous manner across the lactation. The efficiency is evaluated from a residual feed intake derived from a multitrait random regression model, which allows the coefficients to vary over time. This approach allows a dynamic estimation of the traits and their correlations, free from all time-related issues inherent to the traditional methodology, and can easily be adapted and used in a genetic or genomic selection context.

Introduction

With feed costs representing above 50% of the total costs of dairy production (European Commission, 2018), the issue of feed efficiency

has become a priority for the sector. The notion of feed efficiency refers to improving the balance between output (production) and input (feed intake). The most common way to determine feed efficiency in dairy cattle is through residual feed intake (**RFI**). First proposed by Koch et al. (1963), RFI is the difference between the actual feed intake of an animal and its predicted feed intake based on its performance, i.e. the intake necessary to cover the demands of the different energy sinks, estimated by regression. By construction, this method benefits from the RFI being phenotypically independent (or genetically in case of genetic regression) from its predictors, which theoretically allows RFI to reflect digestive and metabolic variabilities (Archer et al., 2002; Berry and Crowley, 2013).

Although this definition of RFI is widely used in dairy cattle (Connor, 2015), some issues remain with this approach. First of all, the time influence on RFI is a key question. RFI is usually measured between two given days. If the trial duration is too short, the number of measures will be low and the results of the prediction will not be accurate. On

the other hand, different biological processes are involved at the different lactation stages, in particular body reserve mobilization in early lactation and reserves accretion associated with pregnancy. Therefore, regression coefficients associated with the different predictors are likely to vary when taken at different lactation stages, leading to a biased assessment of RFI when estimated from point measures over the whole lactation (Li et al., 2017). Moreover, with the advent of precision farming technologies, high-frequency time series measures are becoming available and thereby increasingly offer the opportunity to track efficiency. Key questions in this context are how to deal with changes over time, when to measure RFI and for how long. A second issue comes from the nature of RFI itself. Being a residual, in addition to the actual animal efficiency, it contains all the modeling and measurement errors. Within this context, Fischer et al. (2018) used random regression on the individual level to isolate the cow-specific part of RFI. However, they faced some difficulties due to strong correlations between their predictors (correlations that changed over time during the lactation), which, combined with a limited data set size, restricted their modeling and conclusions.

With the aim of solving these two issues, we investigate in this paper the relationship between intake and its predictors across the lactation using a multitrait random regression model on experimental data. With such a model, based on the variance–covariance functions, it is possible to predict intake from other traits and to compare this prediction with actual intake. This leads to a consistent definition of RFI, with regression coefficients free to vary over time.

Material and methods

Population resources and feeding management

Data were collected between 2002 and 2016 at the Danish Cattle Research Centre (Foulum, Denmark) and shared as part of the GenTORE project (https://www.gentore.eu). Animals were Holstein cows with lactation rank ranging from first to third lactation. Cows were housed in a freestall barn with cubicles and slatted floor and milked in an automatic milking system (**AMS**; DeLaval, Tumba, Sweden) allowing free cow traffic. Data were collected during various trial periods, and cows were not nested within trial. They had *ad libitum* access to a partial mixed ration (**PMR**) varying in nutritional content in accordance with the particular trial in which the cows were involved. The PMR diets used during this period contained the following ingredients (typical g/kg DM): rapeseed meal (106), barley (135), dried sugerbeet pulp (51), grass silage (264), maize silage (422), urea (7) and minerals (18). The corresponding chemical composition was (typical g/kg DM): CP (158), NDF (340), starch (195), sugar (46), crude fat (39), ash (74), with a Net Energy of Lactation (**NEL**) of 6.9 MJ/kg DM. Across the different feeding trials carried out during this period, diet differences did not exceed $+8\%$ of the average value for CP and likewise $+15\%$ for NEL. The nutritional values of the PMR were all within the range of typically recommended requirements, formulated to support the milk yield level of the herd and allocated in amounts allowing approximately 10% orts to ensure *ad libitum* intake. In addition, cows were supplemented daily with a maximum of 3 kg of concentrate to ensure voluntary access to the AMS. These feeding data were already described in previous studies (e.g. Li et al., 2016; Byskov et al., 2017).

Phenotypes and data editing

Data from 1 469 lactations of 740 cows were collected. A weekly measurement of average daily milk yield per cow was obtained from the average of daily milk yield records per cow in each week. Milk samples were taken weekly for analyses of fat and protein. On the same basis, the DM contents in PMR and concentrates were analyzed regularly and the compositions were aligned and merged with feed intake

records to obtain weekly DM intake (**DMI**) values for individual cows. Animals were also automatically weighed at each milking so that BW records were averaged to obtain a weekly record of BW per cow in each week. Body condition score (**BCS**) was evaluated every 2 weeks and scored on a scale from 1 to 5.

A corrected milk (**cmilk**) trait was created following the FAO formula which defines as a standard milk with 4.0% fat and 3.3% protein (FAO [Food and Agriculture Organization of the United Nations], 2010):

$$\text{Corrected milk (kg)} = \text{raw milk (kg)}*(0.337 + 0.116*\text{Fat content}\,(\%)$$

$$+0.06*\text{Protein content}\,(\%))$$

To avoid non-sensical performances, a filter was used to discard records that differed too much from the previous record registered in the same lactation for that animal. Therefore, records implying differences between two consecutive records higher than 12 l for cmilk, 50 kg for the liveweight, 7 kg for the daily DM intake and one unit of BCS were discarded. These threshold values were defined after studying a previous data set with similar performance level. Less than 2% of the data was discarded for cmilk and BCS and about 4% for weight and DMI. A second step of filtering was made on the duration of data collection for each lactation independently: only animals that had been recorded for a minimum duration of 200 days during their lactation were kept. This data set, that was used to conduct the main analyses, contains 40 619 records of cmilk, 40 662 records of weight, 42 177 records of DMI and 19 661 records of BCS, which represents about 73% of the overall records. Additionally, the overall records were used to assess the robustness of our model in case of missing data, and the details of this analysis are presented in Supplementary Material S1.

Statistical analysis

Multitrait random regression model

To analyze the relationship of the four traits along the lactation, a multitrait random regression analysis was performed using the Wombat software (Meyer, 2007). The model used was as follows:

$$y_{ilmr} = c_i + \sum_{n=0}^{3} \beta_{ln}\varphi_{nr}(t) + \sum_{n=0}^{2} \alpha_{mn}\varphi_{nr}(t) + e_{ilmr}$$

where y_{ilmr} is the observation of the rth trait, c_i is the fixed effect of the ith month–year combination corresponding to the record date, β_{ln} is the nth fixed regression coefficient specific to parity class l, α_{mn} is the nth random regression coefficient of the animal m, $\varphi_{nr}(t)$ is the nth coefficient of Legendre polynomial of degree ($d = 2$ for the animal effect and $d = 3$ for the parity class), evaluated at day in milk (DIM) t, and e_{ilmr} is the random residual effect. Residual effects were assumed to have a homogenous residual variance. Successive lactations of the same animal were considered separately, as if they were from different animals, implicitly ignoring any permanent environmental effect across lactations.

From the random part of the equation, we get as outputs the three coefficients of the Legendre polynomial at each time point, as well as the animal solutions. For each trait, we can then obtain at each time and for each cow an estimated animal effect. These animal effects will be denoted $a_{cmilk}(t)$, $a_{weight}(t)$, $a_{DMI}(t)$ and $a_{BCS}(t)$ in the article.

Variance–covariance components of the random regression were obtained and gathered into a 12 by 12 matrix, corresponding to the four traits with three Legendre coefficients each. Variances ($V_{trait1}(t)$) and covariances ($C_{trait1,\,trait2}(t)$) for each time point were estimated by pre- and post-multiplying the variance–covariance matrix by the corresponding time coefficients of the Legendre polynomials using the following formulas:

$$V_{trait1}(t) = [l_0(t) \quad l_1(t) \quad l_2(t)] \begin{bmatrix} \sigma^2_{a_0\,trait1} & \sigma_{a_0a_1\,trait1} & \sigma_{a_0a_2\,trait1} \\ \sigma_{a_0a_1\,trait1} & \sigma^2_{a_1\,trait1} & \sigma_{a_1a_2\,trait1} \\ \sigma_{a_0a_2\,trait1} & \sigma_{a_1a_2\,trait1} & \sigma^2_{a_2\,trait1} \end{bmatrix} \begin{bmatrix} l_0(t) \\ l_1(t) \\ l_2(t) \end{bmatrix}$$

$$C_{trait1,trait2}(t) = [l_0(t) \quad l_1(t) \quad l_2(t)]$$

$$\begin{bmatrix} \sigma_{a_0a_0\,trait1,trait2} & \sigma_{a_0a_1\,trait1,trait2} & \sigma_{a_0a_2\,trait1,trait2} \\ \sigma_{a_0a_1\,trait1,trait2} & \sigma_{a_1a_1\,trait1,trait2} & \sigma_{a_1a_2\,trait1,trait2} \\ \sigma_{a_0a_2\,trait1,trait2} & \sigma_{a_1a_2\,trait1,trait2} & \sigma_{a_2a_2\,trait1,trait2} \end{bmatrix} \begin{bmatrix} l_0(t) \\ l_1(t) \\ l_2(t) \end{bmatrix}$$

These variance–covariance components were then used to calculate correlations between traits across the lactation.

Derivation of residual feed intake

Using elements from the 12 by 12 variance–covariance matrix and the animal effects for cmilk, weight and BCS, it is possible to estimate a predicted intake from the other variables by a matrix regression. If we use the following notation for the variance–covariance matrix at a given time:

$$\begin{bmatrix} V_{DMI} & [C_{DMI,cmilk} \quad C_{DMI,weight} \quad C_{DMI,BCS}] \\ \begin{bmatrix} C_{DMI,cmilk} \\ C_{DMI,weight} \\ C_{DMI,BCS} \end{bmatrix} & \begin{bmatrix} V_{cmilk} & C_{cmilk,weight} & C_{cmilk,BCS} \\ C_{cmilk,weight} & V_{weight} & C_{weight,BCS} \\ C_{cmilk,BCS} & C_{weight,BCS} & V_{BCS} \end{bmatrix} \end{bmatrix} = \begin{bmatrix} \mathbf{B}_{11} & \mathbf{B}_{21} \\ \mathbf{B}_{12} & \mathbf{B}_{22} \end{bmatrix}$$

then a predicted animal effect for DMI can be calculated from a regression similarly to what was proposed by Kennedy et al. (1993), but in its matrix form with:

$$\widehat{a}^*_{eDMI}(t) = \mathbf{B}_{12}(t) * \mathbf{B}_{22}^{-1}(t) * \begin{bmatrix} a_{cmilk}(t) \\ a_{weight}(t) \\ a_{BCS}(t) \end{bmatrix}$$

where $\widehat{a}^*_{eDMI}$ is the predicted animal effect for DMI at time t. Finally, we can construct a RFI estimate which is the difference between the actual animal effect for DMI and the one predicted from the three other variables:

$$RFI(t) = a_{DMI}(t) - \widehat{a}^*_{eDMI}(t)$$

As this RFI is dependent on time, we also defined RFI_{tot} as the averaged RFI of each animal over the whole lactation.

Use of the outputs to study changes between times

One could also consider that changes of BCS are more important than BCS itself in the estimation of RFI. To examine this point, we also estimated $RFI_{\Delta BCS}(t)$ where all the occurrences of BCS-related variables are replaced by ΔBCS-related variables. $\Delta BCS(t)$ is defined as the difference of BCS between two consecutive time points. In the above formulas, it translates as a difference of Legendre polynomial coefficients as follows for the animal effects, the variances and covariances, respectively:

$$a_{\Delta BCS}(t) = [l_0(t) - l_0(t_{-1})] * \widehat{a}_{c0\_BCS} + [l_1(t) - l_1(t_{-1})] * \widehat{a}_{c1\_BCS}$$
$$+ [l_2(t) - l_2(t_{-1})] * \widehat{a}_{c2\_BCS}$$

$$V_{\Delta BCS}(t) = [l_0(t) - l_0(t_{-1}) \quad l_1(t) - l_1(t_{-1}) \quad l_2(t) - l_2(t_{-1})]$$

$$\begin{bmatrix} \sigma^2_{a_0\,BCS} & \sigma_{a_0a_1\,BCS} & \sigma_{a_0a_2\,BCS} \\ \sigma_{a_0a_1\,BCS} & \sigma^2_{a_1\,BCS} & \sigma_{a_1a_2\,BCS} \\ \sigma_{a_0a_2\,BCS} & \sigma_{a_1a_2\,BCS} & \sigma^2_{a_2\,BCS} \end{bmatrix} \begin{bmatrix} l_0(t) - l_0(t_{-1}) \\ l_1(t) - l_1(t_{-1}) \\ l_2(t) - l_2(t_{-1}) \end{bmatrix}$$

$$C_{\Delta BCS,trait2}(t) = [l_0(t) \quad l_1(t) \quad l_2(t)]$$

$$\begin{bmatrix} \sigma_{a_0a_0\Delta BCS,trait2} & \sigma_{a_0a_1\Delta BCS,trait2} & \sigma_{a_0a_2\Delta BCS,trait2} \\ \sigma_{a_1a_0\Delta BCS,trait2} & \sigma_{a_1a_1\Delta BCS,trait2} & \sigma_{a_1a_2\Delta BCS,trait2} \\ \sigma_{a_2a_0\Delta BCS,trait2} & \sigma_{a_2a_1\Delta BCS,trait2} & \sigma_{a_2a_2\Delta BCS,trait2} \end{bmatrix} \begin{bmatrix} l_0(t) - l_0(t_{-1}) \\ l_1(t) - l_1(t_{-1}) \\ l_2(t) - l_2(t_{-1}) \end{bmatrix}$$

And therefore, the predicted animal effect for DMI is calculated from:

$$\widehat{a}^*_{eDMI}(t) = \mathbf{B}_{12}(t) * \mathbf{B}_{22}^{-1}(t) * \begin{bmatrix} a_{cmilk}(t) \\ a_{weight}(t) \\ a_{\Delta BCS}(t) \end{bmatrix}$$

where the $\mathbf{B}$ matrix contains the ΔBCS-related variances and covariances.

The same transformation was also performed on changes of BW.

Results

Descriptive statistics

Descriptive statistics, averaged over the lactation, are reported in Table 1 for the raw data of the four estimated traits, the four animal effects and the RFI. The four traits are variable, with a large range of values. Some values are especially low (e.g. 3.06 kg/d of cmilk or 6.02 kg/d of DM), but they are unlikely measurement errors because, first, data were averaged on a weekly basis and, second, they are relatively close to the previous value for the same animal (due to the filtering step). These extreme values are possibly due to health issues.

The second half of the table presents the animal effects for the four traits estimated from the model and RFI. The animal effect corresponds to the deviation of this animal from the curve fitted to describe the time course of the performance of an average animal for a given trait × parity group, after correction for the other fixed effects. The fixed effects curves are presented in Supplementary Figure S1. For the four traits, the averages of the animal effects obtained from the model are exactly zero, at each time and overall. The statistics presented in Table 1 were obtained only from animal effects for which corresponding raw data existed. By construction, the model can extrapolate and estimate animal effects for each animal at time points beyond the range of data for that animal, even though there was no corresponding raw data. As these extrapolations

Table 1
Means, SD, minima (Min.), maxima (Max.) and 5% and 95% centiles for the raw data of the four considered traits of dairy cattle, animal effects (a_{trait}) and RFI.

Type of data	Trait	Mean	SD	Min.	Max.	5% centile	95% centile
Raw data	Milk (kg of corrected milk)	33.4	7.8	3.1	68.9	21.1	46.9
	Weight (10 kg)[1]	64.3	7.5	39.9	98.3	52.7	77.2
	DMI (kg)	21.7	3.4	6.0	38.7	16.3	27.4
	BCS (scale of 10 to 50)[1]	31.3	3.40	15.0	47.5	25.0	37.5
Computed data	a_{cmilk}	0.20	5.89	−28.61	24.05	−9.12	10.27
	a_{weight}	−0.11	6.75	−22.02	29.91	−10.27	11.65
	a_{DMI}	0.08	2.74	−14.72	12.61	−4.16	4.69
	a_{BCS}	−0.03	2.43	−10.88	13.48	−3.78	3.88
	RFI	0.04	1.37	−18.26	7.16	−1.99	2.21

RFI: Residual Feed Intake.
DMI: DM Intake.
BCS: Body Condition Score.
[1] The original scale was divided (for Weight) or multiplied (for BCS) by 10 in order to have raw data in the multiple trait analysis with similar orders of magnitude for the 4 traits.

were discarded to compute these statistics, it is expected that the means in Table 1 are not exactly zero. The exact same phenomenon occurs for the RFI estimates, with a mean of 0.04.

Correlations between animal effects across time

The correlations between the animal effects for the four traits across the lactation are presented in Fig. 1. The curves of correlation between DMI and cmilk and between DMI and weight showed an increase in early lactation and a decrease in late lactation. The correlations between DMI and cmilk were always higher by 0.1 or 0.2. However, the correlation between cmilk and weight steadily decreased along the lactation up to a point where it became slightly negative at the extreme end of lactation. Animals that produce more milk than average in early lactation are heavier than average, benefiting from a higher intake capacity, but these higher producing animals are less persistent. The correlation between cmilk and BCS was negative throughout the lactation, meaning that those who produce more milk always have a lower BCS than average, and more so in late lactation (they may lose more in early lactation and then have difficulty to rebuild body reserves at the end). The correlation between weight and BCS remained stable over time at around 0.4, reflecting the weight equivalent of a unit BCS (animals having more reserves are heavier at every time point). Finally, the correlation between DMI and BCS started around zero and slowly decreased down to -0.2 in late lactation. At the Legendre polynomial coefficient scale, the correlations are estimated with a high accuracy (averaged SE of 0.04, min = 0.02, max = 0.05), which correspond to variance estimation errors (averaged on the lactation) of below 5% for milk, 1% for BW, 7% for intake and 4% for BCS.

Correlations between animal effects at different time points within each trait are presented in Supplementary Figure S2. The pattern is the same for the four traits with only positive correlations and with higher correlations between animal effects that are close in time. Correlations were always high for weight (>0.8), which is a cumulative trait, while they were a little more variable for the three other traits (correlations >0.4). The largest changes were mostly at the beginning or end of the lactation, and the middle part was very stable (correlations >0.8). These correlations are very accurate with sampling errors being below 4% for intake, 2% for milk and BCS and 1% for BW.

Correlations between the predicted animal effect of DM Intake and the other traits

Correlations between the predicted animal effect for intake ($\widehat{a}^{*}_{eDMI}$) and the four original animal effects were calculated at each time and are presented in Fig. 2. Among the predictors, $\widehat{a}^{*}_{eDMI}$ was very highly correlated

Fig. 2. Correlations between the predicted animal effect for intake ($\widehat{a}^{*}_{eDMI}$) and the four original animal effects (corrected milk (cmilk), weight, DM intake (DMI) and body condition score (BCS)) in the considered dairy herd.

with the animal effect for cmilk, the correlation being always above 0.8 and almost 1 at the beginning of lactation. The second largest correlations were with the animal effect for weight, which starts at 0.7 and slowly decreases down to 0.4 along the lactation. Finally, the correlation with the animal effect for BCS was always very low, starting positive but below 0.2 in early lactation and decreasing down to -0.4 at the end of lactation. The correlation between $\widehat{a}^{*}_{eDMI}$ and a_{DMI} was between 0.6 and 0.8 in early lactation and then remained above 0.8 after 50 days of lactation.

Correlations of the animal effects for all the traits with RFI were also calculated. As expected, by construction, correlations between RFI and the three predictors, as well as with the predicted intake, were zero all along the lactation. The correlation between RFI and a_{DMI} started at almost 0.8 and decreased down to 0.5 during the first to third of lactation and then remained stable until 250 DIM where it slightly increased to 0.6 at the end of lactation.

Evolution of residual feed intake across the lactation

Various individual profiles of RFI across lactation were observed, with some animals being efficient/non-efficient during the whole lactation, while others were efficient in early lactation and non-efficient at the end or the opposite. In order to evaluate the possibility to measure only a part of the lactation and predict the overall RFI, we calculated correlations between RFI at each time and the RFI averaged over the entire lactation (RFI$_{tot}$). Results are presented in Fig. 3. The correlation starts at 0.45, increases up to 0.9 at 92 DIM where it remains stable until 197 DIM, decreasing down to 0.6 afterwards. These results suggests that RFI in the middle of lactation is a good predictor of the mean RFI over the whole lactation.
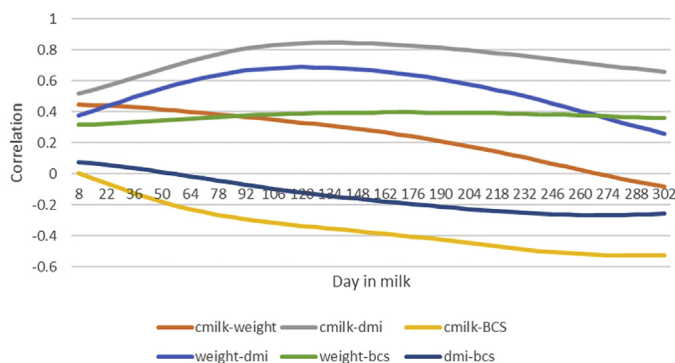

Fig. 1. Time trends of the correlations between the animal effects (i.e. the animal differences from the average at any given time point, across the lactation of dairy cattle) of the corrected milk (cmilk), the body weight, the DM intake (DMI) and the body condition score (BCS).
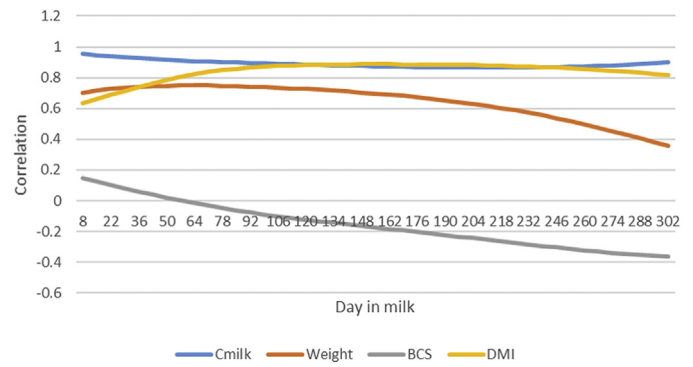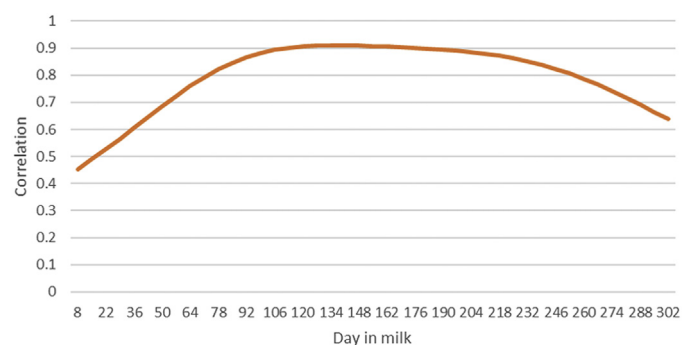

Fig. 3. Correlation between the averaged residual feed intake (RFI) over the whole lactation and the RFI calculated at any given time point in the considered dairy herd.
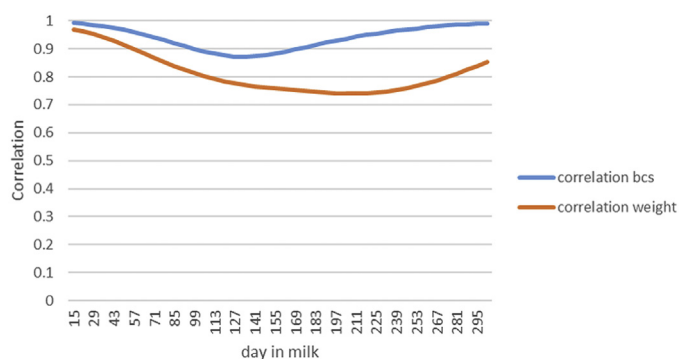
Fig. 4. Correlation between the residual feed intake (RFI) calculated using the trait as a predictor and the RFI calculated using changes of this trait as a predictor, for both weight and body condition score (BCS) in the considered dairy herd.

Using changes rather than the trait itself

Correlations over time points between RFI and $RFI_{\Delta BCS}$ and between RFI and $RFI_{\Delta BW}$ are presented in Fig. 4. Between RFI and $RFI_{\Delta BCS}$, the correlations were high (>0.87), reaching 0.99 both at the beginning and the end of the lactation, indicating that RFI and $RFI_{\Delta BCS}$ are almost the same trait. Between RFI and $RFI_{\Delta BW}$, the correlations are also always high (>0.7), especially in early lactation. This suggests that changes are already taken into account by the use of the three Legendre polynomial coefficients (the changes being the derivatives of the trait). The dramatically high correlation at the beginning (and end of the lactation for BCS) could also indicate that, during these lactation stages, changes are more important than the trait itself, as we do not lose information when considering only changes as predictor. However, this is not true in the middle of lactation where changes are less important and where the traits bring additional values per se.

Discussion

Contribution of the proposed model to the current methodology

In this study, we proposed a modeling strategy which allows the integration of time series data to compute RFI, not only as repeated differences between the end and the beginning of arbitrary periods, but also by considering all traits dynamically. The traditional way of defining RFI – by linear regression – is not appropriate to jointly describe a dairy cow's non-linear trajectories of relevant traits during her entire lactation (Li et al., 2017). Indeed, the allocation of energy varies among the different energy sinks across the lactation, leading to changes in regression coefficients over time. As a result, feed efficiency in dairy cows is usually computed over short time periods (Prendiville et al., 2011; Fischer et al., 2018). This implies that results are based on a small number of data points and therefore are highly sensitive to measurement errors or one-time events (e.g., mastitis). In addition, even on short time periods, RFI estimates may still be subject to approximations due to fixed regression coefficients not being able to reflect biological changes, for instance a switch between loss and gain of body reserves. With the approach presented here, the number of measurements included in the system limits its sensitivity to one-time errors or disturbance. Moreover, it allows a permanent readjustment of the relevant coefficients over time, leading to estimations of RFI that avoid the biases inherent to the traditional RFI estimation methodology. As the model accounts for changes in variance for each trait over time, it also enables the correlations between traits to change over time, giving us a better overview of the relationships between the four traits across lactation.

Various studies already tried to explore further the methodology of RFI or the relationships between its components. For example, a

multitrait random regression model was previously developed by Manzanilla Pech et al. (2014) to investigate the relationship between DMI, milk and live weight over time but RFI was not estimated. Lu et al. (2015) used a modified Cholesky decomposition from a multitrait linear model that allowed greater accuracy in genetic merit prediction in case of partially missing data. Strathe et al. (2014) proposed a RFI derived from a bivariate random regression model for BW and cumulative feed intake in pigs. More recently, Islam et al. (2020) used a Bayesian multivariate random regression to analyze DMI, energy-corrected milk, BW and BCS and derived a genetic RFI from it. The approach presented here is an additional step on the way to improving the modeling of feed efficiency.

Evolution of the correlations between traits across the lactation

The approach used here gives us an overview of the evolution of correlations between traits over the lactation. Such results are still rarely available in the literature where most studies calculated correlations on specific time points, on a fixed period of the lactation, and not in a continuous, dynamic way. The works of Veerkamp and Thompson (1999), Spurlock et al. (2012), Liinamo et al. (2012) and Manzanilla Pech et al. (2014) are some of the rare exceptions. In addition, most of the studies describing correlations include pedigree information and therefore are able to split the animal effect between a genetic component and a permanent environmental effect, which was not our case. Even though the animal effects studied here are not exactly the same as additive genetic effects, some similar patterns with genetic correlations reported in the literature can be observed.

Similarly to what was found here, Manzanilla Pech et al. (2014) reported that genetic correlations within traits across the lactation were generally positive and maximum during mid-lactation and that correlations taken between times further apart were smaller. The very high positive correlations were also in accordance with previous studies (Koenen and Veerkamp, 1998; Veerkamp and Thompson, 1999; and Liinamo et al., 2012 for live weight; Tetens et al., 2014 for DMI; and Veerkamp and Thompson, 1999 and Hüttmann et al., 2009 for milk production). However, Manzanilla Pech et al. (2014) reported slightly negative genetic correlations (-0.2) between milk in early lactation and milk during the rest of the lactation and similarly for DMI, while our correlations between animal effects were always positive.

Correlations observed here for animal effects of the four traits at the same time point were also in general accordance with the literature. High positive genetic correlations were reported by Veerkamp and Thompson (1999) and Spurlock et al. (2012) between DMI and weight. Hüttmann et al. (2009) found that the genetic correlation between these two traits was changing over time with an almost null correlation between 31 and 60 DIM and a correlation of 0.4 between 121 and 180 DIM, while Manzanilla Pech et al. (2014) reported the correlation to be maximum at 34 DIM (0.56) and minimum at 153 DIM (0.29). Our results are showing a pattern similar to what Hüttmann et al. (2009) found, but our correlations are always higher and closer to the values proposed by the other studies. In the literature, general positive genetic correlations (rg) were also reported between milk and DMI (van Elzakker and van Arendonk, 1993: rg = 0.46; Veerkamp and Brotherstone, 1997: rg = 0.34; Vallimont et al., 2010: rg = 0.52). However, Manzanilla Pech et al. (2014) found a negative correlation between milk and DMI in early lactation that become positive and high (>0.7) only after 38 DIM and reached a maximum at 195 DIM (rg = 0.91). Our results are essentially different at the onset of the lactation and similar after. It may be due to differences in animal management around calving or to genetic differences between the data sets. Our evolution of the correlation between milk and weight over time is also very different to what was reported by Manzanilla Pech et al. (2014). These authors found a slightly negative correlation (-0.1) both at the beginning and the end of lactation and a positive correlation of about 0.3 in

mid-lactation. In contrast, our results were similar to what those reported by Karacaören et al. (2006) with a correlation decreasing with time and becoming negative at the end of lactation.

Body condition score is usually not among the traits considered in these kind of studies, and therefore, estimated correlations are scarce. It is expected that cows mobilize body reserves in early lactation, while being in a situation of negative energy balance (Tamminga et al., 1997; Grummer, 2007). In the present study, the negative correlation between animal effects on milk and BCS is in accordance with this as higher than average milk production is associated with lower than average BCS. The stable correlation between weight and BCS supports the suggestion made in previous studies that live weight change can be a good indicator of body reserve mobilization (Thorup et al., 2013; Manzanilla Pech et al., 2014).

Use of the residual feed intake

With the methodology proposed in this paper, we obtain a RFI changing through time for every single animal. This affords new possibilities. First, we identified that the averaged RFI over the lactation is highly correlated with RFI measured in mid-lactation, in accordance with Prendiville et al. (2011) and Connor et al. (2012). If the objective is to identify animals which are on average the most efficient, then the costly measure of intake could be done only during a few weeks during mid-lactation (ideally between 115 and 175 dim based on our results) with only a small loss of information, rather than during the entire lactation.

However, the question of what type of animal is desirable to select for is still valid. Efficient animals on average are also the ones that are more efficient in mid-lactation because this is the longest stable period of the lactation without dramatic changes. But because energy sinks and their relative importance are changing over the lactation, efficient animals in mid lactation are not necessarily the ones which are the most efficient in early or late lactation. It clearly appears that during the lactation, the first 5 to 7 weeks are the most challenging period for the cows, during which they have to face a huge increase of milk production associated with a pronounced mobilization of their body reserves. This pronounced negative energy balance increases the risk of health issues and reduces fertility (Esposito et al., 2014), and it is particularly important not to increase the occurrence of health and fertility issues. Therefore, it is critical to make sure that animals we are selecting for do not have a deep body reserve mobilization in early lactation. Because BCS (and/or its changes) was included in the RFI model, the two traits are phenotypically independent but this may not be the case genetically. In addition, the influence of some health events may have been discarded in the analyses with the data filtering or because animals with dramatic health or reproductive issues were culled. Therefore, the importance of BCS in the overall efficiency (including from an economic point of view) may have been underestimated in the model. The relationship between efficiency and resilience or robustness needs to be further investigated in order to make better choices in selection.

Furthermore, we now get individual trajectories of RFI which are highly variable from one animal to another. An obvious next step would be to perform a cluster analysis to see if we could identify specific types of animals. This could help scientists and breeding companies to determine what is the most suitable type of animals overall.

Other issues and future improvement

If this approach and the associated results are very promising, numerous questions are still to be explored. First, the adaptability of the model in more general situations needs to be tested. For instance, the model should be able to perform well when mixing data from different farms, or when animals are subject to diet changes during the lactation. The diet is indeed a major component of interest when studying feed efficiency because individual digestive efficiency varies with diet composition. This influence of the diet was observed, for example, by Tempelman et al. (2015) and Lu et al. (2017), who determined substantial variability in partial regression coefficients between different rations. Several studies have already addressed this question. Durunna et al. (2011a) and Cassady et al. (2016) found moderate phenotypic correlations of 0.33 and 0.40, respectively, between RFI of young beef cattle determined under both growing and finishing diets. Manafiazar et al. (2015) reported a correlation of 0.30 for RFI between dry-lot conditions and pasture. Estimate of genetic correlations between RFI of the same animals in different situations is rare. They generally give higher values than phenotypic correlations (0.50 in Durunna et al., 2011b; 0.83 in Martin et al., 2019). These results suggest the existence of a genotype-by-environment interaction, both in the case of diet changes and variable systems of production, as discussed by Berry and Crowley (2013) in their review. A test of the ability of the present model to take different diets, or situations, into account is therefore of primary interest.

Then, the mathematical modeling itself could be improved and adapted to situations and data sets. For instance, one could use splines instead of Legendre polynomials for the random regression or a heterogeneous variance for the residual effects.

A main point is also how animals with records for more than one lactation should be taken into account. In its present form, the model considers two lactations from the same animal as being independent, which may have slightly influenced the figures, but without changing any conclusion. As the first aim of this work was to present the method, the focus was not on the RFI of individuals. When the interest is on ranking animals, one can incorporate a between-lactation (permanent environment) animal effect. Moreover, to be more accurate, all relationships among animals should be taken into account when pedigree or genomic information is available, which was not the case here. In addition, pedigree data or genotypes are particularly interesting as it would allow to split the animal effect into a genetic part and a permanent environmental part, as showed by Manzanilla Pech et al. (2014) in a model close to ours. It is important to note that this new way of computing RFI could indeed be used in genetic or genomic evaluations.

Conclusion

We have shown that it is possible to derive RFI from a multitrait random regression model applied to DMI and its predictors, allowing for a dynamic estimation of the traits, free from all time-related issues inherent to the traditional RFI methodology. The model allows a better understanding of the correlations between the predictors during the lactation, and it can be adapted and used in a genetic or genomic selection context.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100101.

Ethics approval

During the experiment, all animals were kept indoors and handled with care, in line with Aarhus University's ethics policy in compliance with the European Union legislation for the protection of animals used for scientific purposes.

Data and model availability statement

None of the data were deposited in an official repository.

Author ORCIDs

Pauline Martin: https://orcid.org/0000-0003-2950-2728.

Author contributions

P. Martin: Conceptualization, Formal analysis, Writing – original draft.
V. Ducrocq: Conceptualization, Methodology, Writing – review & editing.
D.G.M. Gordo: Resources, Writing – review & editing.
N.C. Friggens: Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of interest

The authors declare no conflicts of interest.

References

Archer, J.A., Reverter, A., Herd, R.M., Johnson, D.J., Arthur, P.F., 2002. Genetic variation in feed intake and efficiency of mature beef cows and relationships with postweaning measurements. Proceeding of the 7th world congress on genetic applied to livestock production, 19–23 August 2002, Montpellier, France, pp. 221-225.

Berry, D.P., Crowley, J.J., 2013. Cell biology symposium: genetics of feed efficiency in dairy and beef cattle. Journal of Animal Science 91, 1594–1613.

Byskov, M.V., Fogh, A., Løvendahl, P., 2017. Genetic parameters of rumination time and feed efficiency traits in primiparous Holstein cows under research and commercial conditions. Journal of Dairy Science 100, 9635–9642.

Cassady, C.J., Felix, T.L., Beever, J.E., Shike, D.W., 2016. Effects of timing and duration of test period and diet type on intake and feed efficiency of Charolais-sired cattle. Journal of Animal Science 94, 4748–4758.

Connor, E.E., 2015. Invited review: improving feed efficiency in dairy production: challenges and possibilities. Animal 9, 395–408.

Connor, E.E., Hutchison, J.L., Olson, K.M., Norman, H.D., 2012. TRIENNIAL LACTATION SYMPOSIUM: opportunities for improving milk production efficiency in dairy cattle. Journal of Animal Science 90, 1687–1694.

Durunna, O.N., Mujibi, F.D.N., Goonewardene, L., Okine, E.K., Basarab, J.A., Wang, Z., Moore, S.S., 2011a. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. Journal of Animal Science 89, 158–167.

Durunna, O.N., Plastow, G., Mujibi, F.D.N., Grant, J., Mah, J., Basarab, J.A., Okine, E.K., Moore, S.S., Wang, Z., 2011b. Genetic parameters and genotype × environment interaction for feed efficiency traits in steers fed grower and finisher diets. Journal of Animal Science 89, 3394–3400.

van Elzakker, P.J.M., van Arendonk, J.A.M., 1993. Feed intake, body weight and milk production: genetic analysis of different measurements in lactating dairy heifers. Livestock Production Science 37, 37–51.

Esposito, G., Irons, P.C., Webb, E.C., Chapwanya, A., 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Animal Reproduction Science 144, 60–71.

European Commission, 2018. EU agricultural and farm economics briefs. N°17. EU milk margin index estimate up to 2018. Retrieved on 12 November 2019 from. https://ec.europa.eu/agriculture/sites/agriculture/files/rural-area-economics/briefs/pdf/milk-margin-estimate-2018-no17_en.pdf.

FAO (Food and Agriculture Organization of the United Nations), 2010. Greenhouse gas emissions from the dairy sector: a life cycle assessment. Retrieved on 30 October 2019 from. http://www.fao.org/3/k7930e/k7930e00.pdf.

Fischer, A., Friggens, N.C., Berry, D.P., Faverdin, P., 2018. Isolating the cow-specific part of residual energy intake in lactating dairy cows using random regressions. Animal 12, 1396–1404.

Grummer, R.R., 2007. Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology 68, S281–S288.

Hüttmann, H., Stamer, E., Junge, W., Thaller, G., Kalm, E., 2009. Analysis of feed intake and energy balance of high-yielding first lactating Holstein cows with fixed and random regression models. Animal 3, 181–188.

Islam, M.S., Jensen, J., Løvendahl, P., Karlskov-Mortensen, P., Shirali, M., 2020. Bayesian estimation of genetic variance and response to selection on linear or ratio traits of feed efficiency in dairy cattle. Journal of Dairy Science 103, 9150–9166.

Karacaören, B., Jaffrézic, F., Kadarmideen, H.N., 2006. Genetic parameters for functional traits in dairy cattle from daily random regression models. Journal of Dairy Science 89, 791–798.

Kennedy, B.W., van der Werf, J.H., Meuwissen, T.H., 1993. Genetic and statistical properties of residual feed intake. Journal of Animal Science 71, 3239–3250.

Koch, R.M., Swiger, L.A., Chambers, D., Gregory, K.E., 1963. Efficiency of feed use in beef cattle. Journal of Animal Science 22, 486–494.

Koenen, E.P.C., Veerkamp, R.F., 1998. Genetic covariance functions for live weight, condition score, and dry-matter intake measured at different lactation stages of Holstein Friesian heifers. Livestock Production Science 57, 67–77.

Li, B, Fikse, W.F., Lassen, J., Lidauer, M.H., Løvendahl, P., Mäntysaari, P., Berglund, B., 2016. Genetic parameters for dry matter intake in primiparous Holstein, Nordic red, and Jersey cows in the first half of lactation. Journal of Dairy Science 99, 7232–7239.

Li, B., Berglund, B., Fikse, W.F., Lassen, J., Lidauer, M.H., Mäntysaari, P., Løvendahl, P., 2017. Neglect of lactation stage leads to naive assessment of residual feed intake in dairy cattle. Journal of Dairy Science 100, 9076–9084.

Liinamo, A.-E., Mäntysaari, P., Mäntysaari, E.A., 2012. Short communication: genetic parameters for feed intake, production, and extent of negative energy balance in Nordic red dairy cattle. Journal of Dairy Science 95, 6788–6794.

Lu, Y., Vandehaar, M.J., Spurlock, D.M., Weigel, K.A., Armentano, L.E., Staples, C.R., Connor, E.E., Wang, Z., Bello, N.M., Tempelman, R.J., 2015. An alternative approach to modeling genetic merit of feed efficiency in dairy cattle. Journal of Dairy Science 98, 6535–6551.

Lu, Y., Vandehaar, M.J., Spurlock, D.M., Weigel, K.A., Armentano, L.E., Staples, C.R., Connor, E.E., Wang, Z., Coffey, M., Veerkamp, R.F., de Haas, Y., Tempelman, R.J., 2017. Modeling genetic and nongenetic variation of feed efficiency and its partial relationships between component traits as a function of management and environmental factors. Journal of Dairy Science 100, 412–427.

Manafiazar, G., Basarab, J.A., Baron, V.S., McKeown, L., Doce, R.R., Swift, M., Undi, M., Wittenberg, K., Ominski, K., 2015. Effect of post-weaning residual feed intake classification on grazed grass intake and performance in pregnant beef heifers. Canadian Journal of Animal Science 95, 369–381.

Manzanilla Pech, C.I.V., Veerkamp, R.F., Calus, M.P.L., Zom, R., van Knegsel, A., Pryce, J.E., De Haas, Y., 2014. Genetic parameters across lactation for feed intake, fat- and protein-corrected milk, and liveweight in first-parity Holstein cattle. Journal of Dairy Science 97, 5851–5862.

Martin, P., Taussat, S., Vinet, A., Krauss, D., Maupetit, D., Renand, G., 2019. Genetic parameters and genome-wide association study regarding feed efficiency and slaughter traits in Charolais cows. Journal of Animal Science 97, 3684–3698.

Meyer, K., 2007. WOMBAT—a tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). Journal of Zhejiang University - Science B 8, 815–821.

Prendiville, R., Pierce, K.M., Delaby, L., Buckley, F., 2011. Animal performance and production efficiencies of Holstein-Friesian, Jersey and Jersey × Holstein-Friesian cows throughout lactation. Livestock Science 138, 25–33.

Spurlock, D.M., Dekkers, J.C.M., Fernando, R., Koltes, D.A., Wolc, A., 2012. Genetic parameters for energy balance, feed efficiency, and related traits in Holstein cattle. Journal of Dairy Science 95, 5393–5402.

Strathe, A.B., Mark, T., Nielsen, B., Do, D., Kadarmideen, H.N., Jensen, J., 2014. Deriving genomic breeding values for residual feed intake from covariance functions of random regression models. Proceedings of the 10th world congress on genetic applied to livestock production, 18-22 August 2014, Vancouver, Canada , Abstract 116.

Tamminga, S., Luteijn, P.A., Meijer, R.G.M., 1997. Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livestock Production Science 52, 31–38.

Tempelman, R.J., Spurlock, D.M., Coffey, M., Veerkamp, R.F., Armentano, L.E., Weigel, K.A., de Haas, Y., Staples, C.R., Connor, E.E., Lu, Y., VandeHaar, M.J., 2015. Heterogeneity in genetic and nongenetic variation and energy sink relationships for residual feed intake across research stations and countries. Journal of Dairy Science 98, 2013–2026.

Tetens, J., Thaller, G., Krattenmacher, N., 2014. Genetic and genomic dissection of dry matter intake at different lactation stages in primiparous Holstein cows. Journal of Dairy Science 97, 520–531.

Thorup, V.M., Højsgaard, S., Weisbjerg, M.R., Friggens, N.C., 2013. Energy balance of individual cows can be estimated in real-time on farm using frequent liveweight measures even in the absence of body condition score. Animal 7, 1631–1639.

Vallimont, J.E., Dechow, C.D., Daubert, J.M., Dekleva, M.W., Blum, J.W., Barlieb, C.M., Liu, W., Varga, G.A., Heinrichs, A.J., Baumrucker, C.R., 2010. Genetic parameters of feed intake, production, body weight, body condition score, and selected type traits of Holstein cows in commercial tie-stall barns. Journal of Dairy Science 93, 4892–4901.

Veerkamp, R.F., Brotherstone, S., 1997. Genetic correlations between linear type traits, food intake, live weight and condition score in Holstein Friesian dairy cattle. Animal Science 64, 385–392.

Veerkamp, R.F., Thompson, R., 1999. A covariance function for feed intake, live weight, and Milk yield estimated using a random regression model. Journal of Dairy Science 82, 1565–1573.

Ruminal microbiota is associated with feed-efficiency phenotype of fattening bulls fed high-concentrate diets

S. Costa-Roura[A], D. Villalba [iD][A,D], M. Blanco[B,C], I. Casasús [iD][B,C], J. Balcells [iD][A] and A. R. Seradj [iD][A]

[A]Departament de Ciència Animal, Universitat de Lleida, Avinguda Alcalde Rovira Roure 191, 25198, Lleida, Spain.
[B]Unidad de Producción y Sanidad Animal, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), Avenida Montañana 930, 50059, Zaragoza, Spain.
[C]Instituto Agroalimentario de Aragón – IA2 (CITA-Universidad de Zaragoza), Zaragoza, Spain.
[D]Corresponding author. Email: daniel.villalba@udl.cat

Abstract

Context. Improving feed efficiency in livestock production is of great importance to reduce feeding costs.

Aims. To examine the relationship between ruminal microbiota and variation in feed efficiency in beef cattle fed concentrate-based diets.

Methods. Residual feed intake of 389 fattening bulls, supplied with corn-based concentrate and forage *ad libitum*, was used to estimate animals' feed efficiency. Faeces and ruminal fluid samples, from 48 bulls chosen at random, were collected to estimate their forage intake and to determine their apparent digestibility, ruminal fermentation and microbiota. Those animals with extreme values of feed efficiency (high-efficiency (HE, $n = 12$) and low-efficiency (LE, $n = 13$)) were subjected to further comparisons. Alpha biodiversity was calculated on the basis of the normalised sequence data. Beta diversity was approached through performing a canonical correspondence analysis based on log-transformed sequence data. Genera differential abundance was tested with an ANOVA-like differential expression analysis and genera interactions were determined applying the sparse correlations for compositional data technique.

Key results. No differences in dry matter intake were found between the two categories of feed efficiency ($P = 0.699$); however, HE animals had higher apparent digestibility of dry matter ($P = 0.002$), organic matter ($P = 0.003$) and crude protein ($P = 0.043$). The concentration of volatile fatty acids was unaffected by feed efficiency ($P = 0.676$) but butyrate proportion increased with time in LE animals ($P = 0.047$). Ruminal microbiota was different between HE and LE animals ($P = 0.022$); both α biodiversity and genera network connectance increased with time in LE bulls ($P = 0.005$ for Shannon index and $P = 0.020$ for Simpson index), which suggests that LE animals hosted a more robust ruminal microbiota. Certain genera usually related to high energy loss through methane production were found to establish more connections with other genera in LE animals' rumen than in HE ones. Microbiota function capability suggested that methane metabolism was decreased in HE finishing bulls.

Conclusions. Rumen microbiota was associated with feed efficiency phenotypes in fattening bulls fed concentrate-based diets.

Implications. The possible trade-off between feed efficiency and robustness of ruminal microbiota should be taken into account for the optimisation of cattle production, especially in systems with intrinsic characteristics that may constitute a disturbance to rumen microbial community.

Keywords: apparent digestibility, beef cattle, feed efficiency, rumen microbial community.

Received 5 June 2020, accepted 27 November 2020, published online 28 January 2021

Introduction

Improving feed efficiency (FE) in beef cattle production systems provides an opportunity to cut down on the cost of feeding livestock. In that sense, residual feed intake (RFI) can be used as an index of FE that is independent of variation in bodyweight (BW) and average daily gain (ADG; Arthur *et al.* 2001; Schenkel *et al.* 2004; Arthur and Herd 2008), being the

gold standard index to examine biological mechanisms associated with inter-animal differences in FE. Moreover, some studies have demonstrated the possibility of selection for low RFI as a strategy for greenhouse gas mitigation, as it has been correlated with lower methane emission and greater diet digestibility (Herd and Arthur 2009). Limitations in conducting RFI trials (recording BW and feed intake for a

long time) and searching for rumen microbial markers to identify efficient animals with low RFI have become a contemporary challenge.

Research in cattle has focused mostly on the microbial response to dietary changes and management practices, whereas trials for understanding the relationship between host FE phenotype and rumen microbiota are scarce and yet to be undertaken (Myer *et al*. 2015). Previous studies have shown that rumen microbes are responsible for energy supply through producing organic acids (Huntington 1990), and most taxa associated with variation in FE have been related to cellulolytic, fermentative and metabolic activities (Myer *et al*. 2015). Therefore, differences in the production rate of organic acids lead to variation in nutrient digestibility and fermentation that ultimately change animals' phenotypic efficiency (Herd and Arthur 2009).

This experiment aimed to understand the relationship between ruminal microbiota and variation in FE of beef cattle fed concentrate-based diets.

Materials and methods

Animals, diets and housing

Residual feed-intake data from two feeding experiments comprising 389 fattening bulls were used to explore relationships between ruminal microbiota and FE. This dataset included 317 animals raised at the research facilities of *Cooperativa d'Ivars d'Urgell*, *SCCP* (Ivars d'Urgell, Spain, 41°41′50″N, 0°58′53″E) and 72 animals from the *CITA-La Garcipollera* Research Station (Jaca, Spain, 42°37′34″N, 0°30′10″W). All procedures were performed under Project Licence CEEA 01-07/16 and approved by the in-house Ethics Committee for Animal Experiments at the University of Lleida. Care and use of animals were in accordance with the Spanish Policy for Animal Protection RD 53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Animals raised at the research facilities of *Cooperativa d'Ivars d'Urgell*, *SCCP* were distributed in the following four batches: batches Number 1 to Number 3 included 231 Holstein bulls (63–83 animals per batch) and batch Number 4 included 86 Montbeliard bulls. BW and feed intake data were collected on a daily basis. Animals raised at *CITA-La Garcipollera* Research Station were distributed in the following three batches: batches Number 5 and Number 6 included 28 and 32 Parda de Montaña bulls respectively, and batch Number 7 included 12 Pirenaica bulls. For these animals, BW was measured weekly and feed intake data were collected on a daily basis.

Bodyweight and feed intake data were recorded throughout the entire fattening phase; the first 150 days were considered as the growing phase (121 days old, s.d. 37 days; and 162 kg BW, s.d. 49 kg), followed by a finishing phase, which lasted until animals reached slaughter weight (336 days old, s.d.: 31; and 501 kg BW, s.d. 56 kg).

Animals were fed concentrate and forage *ad libitum*, which were provided separately in two different bunkers, and they had free access to drinking water, following the conventional

beef cattle feeding system in Spain. The concentrates used were very similar in composition and their main ingredients were raw corn, corn gluten feed, raw barley, corn dried distillers grains with solubles and raw chickpea; whereas forage used was mainly barley straw (349 animals), oats haylage (20 animals) and vetch haylage (20 animals). Feed chemical and nutritional composition is shown in Table 1.

Measurements and sampling

Intake of concentrates was recorded automatically at both research facilities, by using automatic feed stations that were equipped with a feedbunk (provided with a scale) and an individual feeder. When a calf entered the feeder, it was identified and its concentrate intake was obtained by difference between initial and final feedbunk weight. Feed stations available at the research facilities of *Cooperativa d'Ivars d'Urgell*, *SCCP* were additionally equipped with a scale under the individual feeder by which the animals were automatically weighed at each visit, whereas at *CITA-La Garcipollera* Research Station, BW data were recorded manually once a week.

Faeces and ruminal fluid samples from 48 bulls (selected at random within batches) were collected twice, at mid-growing period (GRO, 159 days old and 225 kg BW) and mid-finishing period (FIN, 266 days old and 434 kg BW), for forage intake estimation and digestibility, ruminal fermentation and microbial community characterisation.

Faecal excretion and forage intake were calculated on the basis of concentrate intake and adapting the two indigestible-marker system (Owens and Hanson 1992), by using chromium oxide as an external marker and acid insoluble ash as internal marker. Then, apparent digestibility of dry matter (DM), organic matter (OM) and crude protein (CP) were estimated. Detailed information about marker administration, feed and faeces analytical determinations and apparent digestibility calculations have been described in Costa-Roura *et al*. (2020).

Ruminal fluid was sampled in the morning by using an oral stomach tube connected to a vacuum pump. Each sample was

Table 1.   Feed chemical and nutritional composition

Values are means, with minimum and maximum given in parentheses. ADF, acid detergent fibre; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fibre; OM, organic matter; PDIN and PDIE, protein digestible in the small intestine allowed by protein and energy; UFV, forage unit for meat production

Parameter	Concentrate	Forage
Chemical composition (%DM)		
DM (% fresh weight)	87.1 (85.6–87.9)	65.8 (48.9–85.7)
OM	94.6 (94.0–94.8)	88.9 (84.3–92.6)
CP	13.0 (11.2–14.0)	11.2 (7.2–16.4)
EE	4.2 (2.5–7.2)	2.0 (1.4–2.7)
NDF	16.6 (13.5–20.7)	57.1 (44.2–75.5)
ADF	5.9 (4.7–7.9)	33.5 (28.2–43.8)
Nutritional composition		
UFV (UFV/kg DM)	1.02 (0.97–1.03)	0.55 (0.36–0.73)
PDIN (g/kg DM)	91.8 (79.8–95.9)	65.4 (40.8–93.7)
PDIE (g/kg DM)	87.4 (80.3–94.9)	56.0 (52.6–58.0)

obtained through two sequential collections. First, ruminal fluid (~200 mL) was collected and discarded to avoid sample contamination with saliva that could get into the tube during its introduction through the animal's mouth and oesophagus. After that, ruminal fluid (~200 mL) was re-extracted, strained through a cheesecloth and its pH was recorded (Testo 205, Testo AG, Germany). Then, ruminal fluid was sampled for DNA extraction, and determination of ammonia-nitrogen (N) and volatile fatty acid (VFA) concentrations, and immediately frozen on dry ice. Sample preservation conditions and analytical procedures for ammonia-N and VFA determination are detailed in Costa-Roura *et al*. (2020).

Extraction and sequencing of DNA

Extraction of DNA was performed on freeze-dried ruminal fluid (the initial amount of the sample was 60 mg) through physical disruption (1 min) by using a bead beater (Mini-bead Beater 1, BioSpec Products, USA) and subsequent DNA purification was performed with the QIAamp DNA Stool Mini Kit (ID: 51504; QIAGEN N.V., Germany), with the modifications of greater temperature (95°C) and greater elution time (3 min) to ensure maximum DNA concentration in the final elute. Amplification of DNA was performed by using primers 341F and 805R, which target the V3 and V4 regions of the bacterial and archaeal 16S rRNA. Sequencing was conducted on an Illumina MiSeq 2x300 platform by Era7 Bioinformatics (Spain).

Assembly and filtration of sample reads, as well as operational taxonomic unit (OTU) preparation have been detailed in Costa-Roura *et al*. (2020).

Estimation of RFI and clustering

Weight data were fitted to a third-degree polynomials model in function of age (Eqn 1) that allows the estimation of the ADG of each animal at any age.

$$\text{Weight}_{i,\,age} = \sum_{j=0}^{j=3} \left(b_{\text{BATCH},j} + A_{i,j} \right) \cdot age_j + e_{i,age} \quad (1)$$

where $b_{\text{BATCH},j}$ is the batch effect (fixed); $A_{i,j,}$ is the *j*th random coefficient for the *i*th animal effect; age is the age of the animal (days) and $e_{i,age}$ the residual term.

Thereafter, ADG_dev was obtained as the first derivative of Eqn 1 for each month by using the monthly average age of each animal (Eqn 2). The individual ADG deviation (ADG_dev) will account for the difference of growth of the animal compared with the average of the batch at each age.

$$\text{ADG\_dev}_{i,age} = \sum_{j=1}^{j=3} j \times A_{i,j} \cdot \ age^{(j-1)} \quad (2)$$

In total, 86 records (3%) out of three standard deviations of the mean were considered as outliers and excluded from the dataset.

Residual feed intake was modelled (Eqn 3) using the random regression coefficient approach proposed by Savietto *et al*. (2014). The model included batch, age (months), ADGdev,

metabolic weight (MW; monthly mean $BW^{0.64}$) and was defined as follows:

$$FI_{ij} = B_{0,\text{animal i}} + \text{Batch} \times age_j + (\text{Batch} + B_{1,\text{animal i}})$$
$$\times MW_{ij} + (\text{Batch} + B_{2,\text{animal i}}) \times \text{ADG\_dev}_{ij} + e_{ij} \quad (3)$$

where FI_{ij} is DM intake measured for Animal *i* in Month *j* and $B_{k,\,\text{animal i}}$ are the random coefficients for animal effect modelled using an unstructured matrix of variances between them.

The inclusion of batch effect in Eqn 1 and Eqn 3 assured that the FE calculated was not affected by diet differences.

On the basis of the individual coefficients of ADG ($B_{1,\,\text{animal i}}$) and MW ($B_{2,\,\text{animal i}}$), animals were segregated into four categories of FE, as follows:
(1) Animals with positive coefficients of both ADG and MW belonged to 'low-efficiency in ADG and low-efficiency in MW' category.
(2) Animals with positive coefficient of ADG but negative coefficient of MW belonged to 'low-efficiency in ADG and high-efficiency in MW' category.
(3) Animals with negative coefficient of ADG but positive coefficient of MW belonged to 'high-efficiency in ADG and low-efficiency in MW' category.
(4) Animals with negative coefficients of both ADG and MW belonged to 'high-efficiency in ADG and high-efficiency in MW' category.

For the purpose of the present study, the two extreme categories (1 and 4) were considered as high-efficiency (HE, positive RFI) and low-efficiency (LE, negative RFI) animals, respectively. This clustering (HE vs LE) was subjected to bioinformatic analyses of apparent digestibility, ruminal fermentation and microbiota data as explained below.

Bioinformatics

Sequence data were normalised and α biodiversity indices were calculated to measure the variability of OTUs within a sample (R Core Team 2020, Vegan package).

To measure differences in microbiota composition among samples, β diversity was approached through performing a canonical correspondence analysis, based on log-transformed OTU data (zeros were replaced by adding 1 to each value), and including FE (HE vs LE), period (GRO vs FIN) and both ADG and MW coefficients as explanatory variables (R Core Team 2020, Vegan package).

To circumvent the compositional bias problem (Tsilimigras and Fodor 2016; Gloor *et al*. 2017; Calle 2019), we applied the Aitchison's centred log ratio (clr) transformation to carry the data to a Euclidean space, after replacing zeros by adding 1 to each value. So as to test the significance of the following effects: FE (HE vs LE), period (GRO vs FIN) and both ADG and MW coefficients on microbiota composition, a permutational multivariate analysis of variance (Adonis) was conducted on the basis of the clr Euclidean distance and calculating statistical significance after 10 000 random permutations (R Core Team 2020, Vegan package). So as to decipher which genera abundance were responsible for the differences among groups, an ANOVA-like differential

expression (ALDEx) analysis was conducted over those genera present at least at 50% of the individuals (R Core Team 2020, Aldex2 package; Fernandes *et al*. 2013). Finally, to describe the interactions within rumen microbial community, we performed a network analysis through sparse correlations for compositional data (SparCC) technique (R Core Team 2020, SpiecEasi package; Friedman and Alm 2012) over those genera present at least at 50% of the individuals. Microbial networks were graphically represented (R Core Team 2020; igraph package) and their complexity was described in terms of number of nodes (genera), number of edges (significant positive or negative correlations), node degree (number of connections that any node establishes with other nodes) and betweenness (measure of centrality in a graph based on shortest paths).

Microbiota functional content was assessed using a topic model approach (R Core Team 2020; themetagenomics package) that consists on (1) capturing groups of co-occurring taxa termed 'topics', (2) uncovering within-topic functional potential, and (3) linking these topics and their functional content to specific sample features (e.g. FE phenotypes; Woloszynek *et al*. 2019).

Statistical analyses

The models of RFI were solved using MIXED procedure of SAS statistical software (SAS v9.4, Cary, NC, USA). Intake, apparent digestibility, ruminal fermentation parameters and microbial α biodiversity data were analysed with a mixed model, including FE (HE vs LE), period (GRO vs FIN) and

their interaction as fixed effects and animal as a random effect, to account for repeated measurements (R Core Team 2020, lme4 package). Differences among least square means were assessed using Tukey multiple-comparison test (R Core Team 2020, emmeans package). Individual samples out of three standard deviations of the mean were discarded and not included in the statistical analysis. Results are reported as least square means and standard error of mean. Significant effects were declared at $P < 0.05$ and tendency to difference at P between 0.05 and 0.10.

Results

The four defined FE categories based on random regression coefficients included between 85 and 108 animals each; the two extreme categories corresponding to HE and LE animals had statistically different means for ADG and MW coefficients (Table S1, available as Supplementary Material to this paper). The set of 48 bulls that were sampled for apparent digestibility, ruminal fermentation and microbiota characterisation were equally distributed within the four categories.

The FE by period interactions were not significant for any of the response variables measured in the present study; thus, only the main effects means are presented and discussed.

Intake, apparent digestibility and ruminal fermentation parameters

Data on DM intake (Table 2) indicated that bulls' concentrate and forage intakes were similar between FE categories (HE vs LE). However, animals classified as HE had greater apparent

Table 2.   Dry matter (DM) intake, nutrient apparent digestibility and ruminal fermentation parameters

Obtained in intensively reared bulls in the following two periods: growing (GRO: 159 days old and 225 kg bodyweight) and finishing (FIN: 266 days old and 434 kg bodyweight). Residual feed intake was modelled to classify animals into two categories of feed efficiency, namely, high efficiency (HE, n = 12) and low efficiency (LE, n = 13). Standard error of the mean (s.e.m.) and significance of feed efficiency and period effects are shown. No feed efficiency by period interaction was statistically significant ($P > 0.05$) and these are not included in the table. A : P, acetate-to-propionate ratio; CP, crude protein; N, nitrogen; OM, organic matter; VFA, volatile fatty acids. Mean values within a row followed by different letters differ significantly (at $P = 0.05$)

Parameter	Feed efficiency		Period		s.e.m.	P-value	
	HE	LE	GRO	FIN		Feed efficiency	Period
Intake							
DM (kg/day)	7.52	7.64	5.88b	9.28a	0.235	0.699	<0.001
Concentrate DM	6.40	6.59	5.28b	7.72a	0.191	0.479	<0.001
Forage DM	1.09	0.93	0.60b	1.42a	0.137	0.397	<0.001
Apparent digestibility coefficients (%)							
DM	75.16a	70.82b	72.88	73.09	0.955	0.002	0.875
OM	76.15a	71.94b	73.84	74.25	0.995	0.003	0.760
CP	71.53a	68.52b	69.64	70.41	1.020	0.043	0.579
Ruminal fermentation parameters							
pH	6.93	6.97	7.16a	6.75b	0.080	0.710	<0.001
Ammonia-N (mg/L)	12.36	19.27	12.03	19.59	3.618	0.181	0.095
VFA (mmol/L)	70.87	74.41	68.22	77.05	6.102	0.676	0.183
VFA (%)							
Acetate	49.63	51.42	49.32	51.73	1.532	0.403	0.093
Propionate	37.70	35.88	38.89a	34.69b	1.867	0.487	0.015
Butyrate	8.18	8.38	7.70b	8.86a	0.425	0.737	0.025
Branched-chain VFA	1.93	1.99	1.66b	2.27a	0.128	0.724	<0.001
Ratio A : P	1.42	1.58	1.30b	1.69a	0.148	0.431	0.017

digestibility coefficients of DM, OM and CP than did their LE counterparts.

Data on ruminal fermentation parameters (Table 2) showed no differences in ruminal pH between HE and LE bulls. Ammonia-N concentration was low and variable among animals; therefore, no statistical differences between FE categories were found. Although the total VFA concentration remained unaffected by FE, numerical differences were found in molar proportions of the main VFA; HE animals had a lower proportion of acetate and a higher proportion of propionate than did LE ones. Butyrate proportion increased with time in the case of LE animals (7.46% vs 9.30% for GRO and FIN periods, respectively; $P = 0.047$) whereas it remained equal for HE animals (7.95% vs 8.42% for GRO and FIN, respectively; $P = 0.908$). In contrast, branched-chain VFA proportion (isobutyrate and isovalerate) increased with time only in the case of HE bulls (1.55% vs 2.31% for GRO and FIN, respectively; $P = 0.006$).

Microbial dataset features

Sequencing procedure yielded an average of (mean $\pm$ s.e.m.) 19 862 $\pm$ 2215 sequences per sample, resulting in 973 259 sequences in the whole study. In total, 787 OTUs were obtained at the 98% sequence-similarity cut-off levels, with 114 $\pm$ 5 as the mean number of OTUs per sample. Good's coverage value was 99.69 $\pm$ 0.03%, suggesting that more than 99% of bacterial and archaeal phylotypes were identified. The unclassified rate of OTUs at genus level was 0.75 $\pm$ 0.09%. Shared OTUs by all individuals in each FE category and period were deemed to be core bacterial/archaeal communities. Core community gathered 69.90 $\pm$ 2.94% of analysed sequences and was composed of five OTUs, namely, *Prevotella ruminicola*, unclassified *Prevotella* (both representing more than 84% of shared sequences), unclassified *Roseburia*, *Sharpea azabuensis* and unclassified *Methanobrevibacter*.

Microbial community biodiversity

Alpha biodiversity (Table 3) was found to be similar among bulls differing in their FE; however, Shannon and Simpson index values increased with time only in LE animals (Shannon index 1.51 vs 2.13, $P = 0.005$; Simpson index 0.53 vs 0.70, $P = 0.020$, for GRO and FIN, respectively).

Beta biodiversity is graphically represented in Fig. 1, as well as the effects of explanatory variables included in the model, namely, FE, period, and both MW and ADG coefficients in the RFI model. Samples are clearly clustered by period and FE, with the effects of MW and ADG coefficients being less graphically evident. Adonis test results confirmed the foreseen differences in ruminal microbiota composition when comparing sampling periods (GRO vs FIN, $P < 0.001$), FE categories (HE vs LE, $P = 0.022$) and MW coefficient values ($P = 0.021$), but not in the case of ADG coefficient values ($P = 0.276$). Statistical differences in abundance of genera between FE categories (HE vs LE) could not be detected by ALDEx analysis, regardless of the sampling period (Fig. S1, available as Supplementary Material to this paper).

Microbial network

Microbial networks were built to test interactions among bacterial and archaeal genera (Fig. 2). Degree of interaction
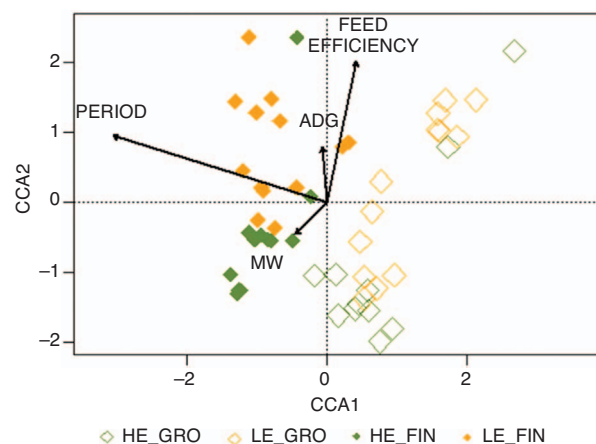

Fig. 1. Graphical representation of canonical correspondence analysis (CCA) on bacterial and archaeal operational taxonomic units (OTUs) in ruminal fluid, obtained in intensively reared bulls in the following two periods: growing (GRO: 159 days old and 225 kg bodyweight) and finishing (FIN: 266 days old and 434 kg bodyweight). Residual feed intake was modelled to classify animals into two categories of feed efficiency, namely, high efficiency (HE) and low efficiency (LE). The analysis included feed efficiency, period, and both average daily gain (ADG) and metabolic weight (MW) coefficients as explanatory variables.

Table 3.   Ruminal microbial α biodiversity

Obtained in intensively reared bulls in the following two periods: growing (GRO: 159 days old and 225 kg bodyweight) and finishing (FIN: 266 days old and 434 kg bodyweight). Residual feed intake was modelled to classify animals into two categories of feed efficiency, namely, high efficiency (HE, n = 12) and low efficiency (LE, n = 13). Standard error of the mean (s.e.m.) and significance of feed efficiency and period effects are shown. No feed efficiency by period interaction was statistically significant ($P > 0.05$) and these are not included in the table. Mean values within a row followed by different letters differ significantly (at $P = 0.05$)

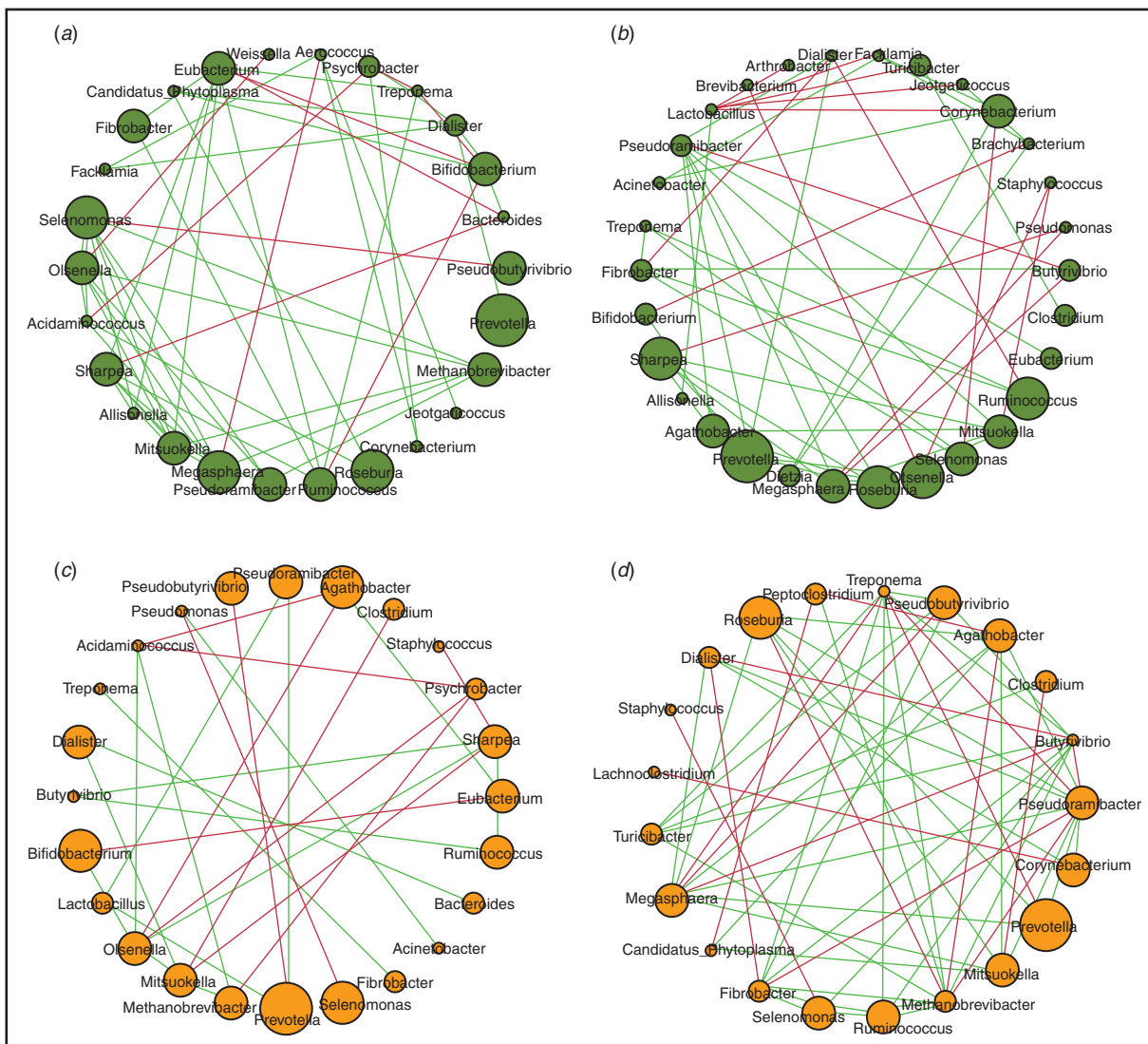| Parameter | Feed efficiency | | Period | | s.e.m. | *P*-value | |
	HE	LE	GRO	FIN		Feed efficiency	Period
Shannon index	1.76	1.82	1.53b	2.05a	0.149	0.760	<0.001
Simpson index	0.61	0.61	0.54b	0.68a	0.050	0.942	0.002
Richness	101.10	107.69	96.87b	111.92a	5.824	0.408	0.072

Fig. 2. Bacterial and archaeal genera network in the rumen of intensively reared fattening bulls: (*a–c*) GRO: 159 days old and 225 kg bodyweight; (*b–d*) FIN: 266 days old and 434 kg bodyweight. Residual feed intake was modelled to classify animals into two categories of feed efficiency, namely, (*a, b*) high efficiency (HE) and (*c, d*) low efficiency (LE). Networks were generated on the basis of those genera that established significant correlations (*r* > 0.60 and *P* < 0.05). Green and red edges indicate positive and negative correlations, respectively. Node size is proportional to genus abundance in the ruminal fluid.

was studied through the number of genera (nodes) that established significant interactions (edges) with other genera, as well as the number of interactions established per node (node degree). During the growing period, HE bulls had similar number of nodes taking part in the microbial network as did LE bulls (26 in HE vs 24 in LE), but higher number of edges (57 in HE vs 28 in LE) and a higher average node degree (4.38 in HE vs 2.33 in LE). During the finishing period, microbial network architecture changed; HE bulls continued to have more correlating nodes than did LE bulls (30 in HE vs 21 in LE), but LE animals drastically increased their number of edges (53 in HE vs 59 in LE) and the node degree (3.53 in HE vs 5.62 in LE).

Moreover, we investigated microbial genera that act as main information gateways in networks in terms of

betweenness centrality, i.e. the extent to which one node lies on paths that connect other nodes. Networks of HE animals presented higher betweenness centrality than did those of LE animals in growing (3.88 in HE vs 1.04 in LE) but not in finishing (2.37 in HE vs 2.52 in LE) period.

Microbial functional capability

After predicting functional content of ruminal microbiota, two pathways were found to be differentially expressed depending on the animal FE phenotype. In the growing period, ABC transporter pathway (ATP-dependent transport of molecules across cell membrane) was more active in HE animals than in LE animals, and, in the finishing period, methane metabolism

was downregulated in the rumen of HE individuals when compared with LE animals.

Discussion

RFI and mechanisms underlying the variability of FE

Variations in RFI occur due to potential physiological mechanisms such as digestion, fermentation and metabolism (Herd and Arthur 2009). Our findings showed that HE animals apparently digested more feed, in terms of DM, OM and CP, than did LE ones. These results are in accordance with previous studies in which more efficient animals showed a higher nutrient digestibility and a smaller nutrient loss through waste and methane emission (Richardson *et al*. 1996; Nkrumah *et al*. 2006). Negesse *et al*. (2017) also observed improved apparent digestibility coefficients of DM, OM and CP in HE heifers; these animals excreted a smaller proportion of N through faeces and their N biological value (digestible N ratio) was higher than that of less efficient heifers, suggesting that CP digestion and metabolism may be enhanced in HE animals. In comparison, de Assis Lage *et al*. (2019) did not find differences in digestibility coefficients of such nutrients but they reported a tendency of HE heifers to better digest ether extract fraction.

Volatile fatty acids are products of rumen microbial fermentation of carbohydrates, constituting the main energy source for ruminants (Bergman 1990). Although differences between HE and LE bulls did not reach significance for any ruminal fermentation parameter, numerical values indicated that LE animals had a fermentation pattern oriented towards the production of higher molar proportion of acetic and less propionic acids than did HE animals, with the consequent effect on acetate-to-propionate ratio. These observed differences in rumen fermentation pattern may be playing a role in the bulls' FE phenotype, since metabolic hydrogen produced in the first step of acetic acid pathway is later taken up by methanogens, increasing energy loss through gas emissions (Ungerfeld 2020).

During the growing period, molar proportions of the major VFA were similar to those observed by Yuste *et al*. (2020) in beef heifers fed a similar *ad libitum* concentrate plus straw diet. However, during the finishing period, the higher amount of total VFA, concomitant with significantly lower ruminal pH, were rooted in the increased DM intake and, consequently, in the higher extent of fermentation process. However, propionic acid showed a different trend and it was higher in younger animals. Hernandez-Urdaneta *et al*. (1976) reported that forage-to-concentrate ratio affects the molar proportions of VFA, which for high-concentrate diets changes towards decreased acetic and increased propionic acid; therefore, in our experiment, the lower forage-to-concentrate ratio during the growing (11%) than in the finishing period (18%) can explain the observed decreased proportion of propionic acid with time.

RFI and ruminal microbial community

In the present study, Illumina sequencing technology was used to analyse bacterial and archaeal composition, biodiversity, connectance and functional capability within the rumen of intensively reared bulls differing in their FE.

A negative correlation between ruminal microbial α biodiversity and FE has been previously described in milking cows (Shabat *et al*. 2016), suggesting that efficient microbiotas are less complex but more specialised in providing higher concentrations of relevant output metabolites that can be used to meet host's energy requirements. In a similar manner, our results showed that microbial α diversity values significantly increased with time only in LE bulls. Microbial diversity is also positively correlated with community stability and robustness, as both differential response to variable conditions and functional redundancy of species are enhanced (McCann 2000; Moya and Ferrer 2016). Thus, it seems reasonable to hypothesise that α biodiversity of the microbiota has positive and negative coexisting effects on ecosystem robustness and feed utilisation efficiency, respectively. The fact that LE bulls considerably increased their genera network connectance with time, while HE bulls kept it constant or even diminished it, supports the hypothesis that LE animals' ruminal microbiota could be more robust and have an enhanced ability to cope with possible disturbances (Dunne *et al*. 2002).

Even though β diversity representation showed clear clustering of bulls' microbial community, no statistical differences in the abundance of main genera could be found between FE categories. Considering that some studies have had success in reporting a relationship between certain microbial taxa and animal's FE (McCann *et al*. 2014; Myer *et al*. 2015; Perea *et al*. 2017; Delgado *et al*. 2019), we consider that the following factors could hinder detection of such relationship: (1) there can be substantial animal-to-animal variation in the rumen microbial community, thus requiring a greater number of animals to be able to observe a significant association between microbial taxa and FE (Brulc *et al*. 2009; Weimer *et al*. 2010), and (2) the lack of differences observed between FE categories at the main-genera level may indicate that the important variation in microbial communities lies at a finer resolution (e.g. at species level or low-abundance genera).

Kittelmann *et al*. (2014) described the existence of three ruminal microbial communities linked to different methane yields in sheep; ruminotype H was characterised by the highest methane emissions and harboured the higher abundance of species belonging to *Ruminococcus*, other Ruminococcaceae, Lachnospiraceae, Catabacteriaceae, *Coprococcus*, other Clostridiales, *Prevotella*, other Bacteroidales and Alphaproteobacteria. In a recent study in sheep, Ghanbari Maman *et al*. (2020) also identified certain genes from Lachnospiraceae, *Ruminococcus*, *Butyrivibrio* and *Selenomonas* taxa that can have significant effects on methane production pathway. In accordance with these studies, our co-abundance analysis showed that certain genera previously related to high methane emission (e.g. *Methanobrevibacter*, *Roseburia*, *Agathobacter*, *Butyrivibrio*, *Pseudobutyrivibrio*, *Ruminococcus*, *Selenomonas*) either were more central or evolved to be more central in LE animal networks during the transition from growing to finishing periods (Tables S2, Table S3, available as Supplementary Material to this paper), which could at least partially cause their lower FE.

Recent studies have highlighted a possible relationship between microbial metabolic functions and the animal's FE, but the nature of such relationship is still unclear. Li *et al*. (2016) observed that HE cattle had a more active metabolism of nucleotides, as well as of various energy-generating molecules (e.g. propanoate, glyoxylate and dicarboxylate, starch and sucrose), hypothesising that such increased metabolic activity could enhance feed digestion and provide the host with more nutrients. Li *et al*. (2016) and Elolimy *et al*. (2020) also reported that rumen microbiota of the most efficient cattle was more active in cell proliferation and survivability, inducing cellular growth and increasing tolerance to viral infection; likewise, our results showed enhanced cell membrane transport functions in HE growing animals. Finally, the observed decrease of methane metabolism activity in HE finishing bulls (Shabat *et al*. 2016) supports the previous idea that high and low methane emitters can have a similar abundance of ruminal methanogens but differential expression and transcription of methanogenesis pathway genes (Shi *et al*. 2014).

Conclusions

The exploration of the relationship between rumen microbial community and host FE showed increased nutrient digestibility in HE animals. Alpha biodiversity and genera network connectance increased with time in LE bulls, highlighting a possible trade-off between FE and ruminal microbiota robustness. Moreover, certain genera that have previously been related to high methane emission were more central in LE animals' genera networks. Our results have provided evidence that the rumen microbiota could be one of the biological factors associated with variation in cattle FE.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

Arthur JPF, Herd RM (2008) Residual feed intake in beef cattle. *Revista Brasileira de Zootecnia* **37**, 269–279. doi:10.1590/S1516-3598 2008001300031

Arthur PF, Archer JA, Johnston DJ, Herd RM, Richardson EC, Parnell PF (2001) Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. *Journal of Animal Science* **79**, 2805–2811. doi:10.2527/2001.79112805x

Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* **70**, 567–590. doi:10.1152/physrev.1990.70.2.567

Brulc JM, Antonopoulos DA, Berg Miller ME, Wilson MK, Yannarell AC, Dinsdale EA, Edwards RE, Frank ED, Emerson JB, Wacklin P, Coutinho PM, Henrissat B, Nelson KE, White BA (2009) Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 1948–1953. doi:10.1073/pnas.0806191105

Calle ML (2019) Statistical analysis of metagenomics data. *Genomics & Informatics* **17**, e6. doi:10.5808/GI.2019.17.1.e6

Costa-Roura S, Balcells J, de la Fuente G, Mora-Gil J, Llanes N, Villalba D (2020) Nutrient utilization efficiency, ruminal fermentation and microbial community in Holstein bulls fed concentrate-based diets with different forage source. *Animal Feed Science and Technology* **269**, 114662. doi:10.1016/j.anifeedsci.2020.114662

de Assis Lage CF, Gesteira Coelho S, Diniz Neto H do C, Rocha Malacco VM, Pacheco Rodrigues JP, Sacramento JP, Samarini Machado F, Ribeiro Pereira LG, Ribeiro Tomich T, Magalhães Campos M (2019) Relationship between feed efficiency indexes and performance, body measurements, digestibility, energy partitioning, and nitrogen partitioning in pre-weaning dairy heifers. *PLoS One* **14**, e0223368. doi:10.1371/journal.pone.0223368

Delgado B, Bach A, Guasch I, González C, Elcoso G, Pryce JE, Gonzalez-Recio O (2019) Whole rumen metagenome sequencing allows classifying and predicting feed efficiency and intake levels in cattle. *Scientific Reports* **9**, 11. doi:10.1038/s41598-018-36673-w

Dunne JA, Williams RJ, Martinez ND (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters* **5**, 558–567. doi:10.1046/j.1461-0248.2002.00354.x

Elolimy A, Alharthi A, Zeineldin M, Parys C, Loor JJ (2020) Residual feed intake divergence during the preweaning period is associated with unique hindgut microbiome and metabolome profiles in neonatal Holstein heifer calves. *Journal of Animal Science and Biotechnology* **11**, 13. doi:10.1186/s40104-019-0406-x

Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB (2013) ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-seq. *PLoS One* **8**, e67019. doi:10.1371/journal.pone.0067019

Friedman J, Alm EJ (2012) Inferring correlation networks from genomic survey data. *PLoS Computational Biology* **8**, e1002687. doi:10.1371/journal.pcbi.1002687

Ghanbari Maman L, Palizban F, Fallah Atanaki F, Elmi Ghiasi N, Ariaeenejad S, Ghaffari MR, Hosseini Salekdeh G, Kavousi K (2020) Co-abundance analysis reveals hidden players associated with high methane yield phenotype in sheep rumen microbiome. *Scientific Reports* **10**, 4995. doi:10.1038/s41598-020-61942-y

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ (2017) Microbiome datasets are compositional: and this is not optional. *Frontiers in Microbiology* **8**, 2224. doi:10.3389/fmicb.2017.02224

Herd RM, Arthur PF (2009) Physiological basis for residual feed intake. *Journal of Animal Science* **87**, E64–E71. doi:10.2527/jas.2008-1345

Hernandez-Urdaneta A, Coppock CE, McDowell RE, Gianola D, Smith NE (1976) Changes in forage-concentrate ratio of complete feeds for dairy cows. *Journal of Dairy Science* **59**, 695–707. doi:10.3168/jds.S0022-0302(76)84260-9

Huntington G (1990) Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reproduction, Nutrition, Development* **30**, 35–47. doi:10.1051/rnd:19900103

Kittelmann S, Pinares-Patiño CS, Seedorf H, Kirk MR, Ganesh S, McEwan JC, Janssen PH (2014) Two different bacterial community types are linked with the low-methane emission trait in sheep. *PLoS One* **9**, e103171. doi:10.1371/journal.pone.0103171

Li F, Zhou M, Ominski K, Guan LL (2016) Does the rumen microbiome play a role in feed efficiency of beef cattle? *Journal of Animal Science* **94**, 44–48. doi:10.2527/jas.2016-0524

McCann KS (2000) The diversity–stability debate. *Nature* **405**, 228–233. doi:10.1038/35012234

McCann JC, Wiley LM, Forbes TD, Rouquette FM, Tedeschi LO (2014) Relationship between the rumen microbiome and residual feed intake-efficiency of Brahman bulls stocked on bermudagrass pastures. *PLoS One* **9**, e91864. doi:10.1371/journal.pone.0091864

Moya A, Ferrer M (2016) Functional redundancy-induced stability of gut microbiota subjected to disturbance. *Trends in Microbiology* **24**, 402–413. doi:10.1016/j.tim.2016.02.002

Myer PR, Smith TPL, Wells JE, Kuehn LA, Freetly HC (2015) Rumen microbiome from steers differing in feed efficiency. *PLoS One* **10**, e0129174. doi:10.1371/journal.pone.0129174

Negesse T, Datt C, Kundu SS (2017) Residual feed intake, digestibility of nutrients and efficiency of water utilizations in Murrah buffalo heifers. *Journal of Dairy, Veterinary & Animal Research* **5**, 74–80. doi:10.15406/jdvar.2017.05.00138

Nkrumah JD, Okine EK, Mathison GW, Schmid K, Li C, Basarab JA, Price MA, Wang Z, Moore SS (2006) Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. *Journal of Animal Science* **84**, 145–153. doi:10.2527/2006.841145x

Owens FN, Hanson CF (1992) External and internal markers for appraising site and extent of digestion in ruminants. *Journal of Dairy Science* **75**, 2605–2617. doi:10.3168/jds.S0022-0302(92)78023-0

Perea K, Perz K, Olivo SK, Williams A, Lachman M, Ishaq SL, Thomson J, Yeoman CJ (2017) Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota. *Journal of Animal Science* **95**, 2585–2592. doi:10.2527/jas2016.1222

R Core Team (2020) 'R: a language and environment for statistical computing.' (R Foundation for Statistical Computing: Vienna, Austria)

Richardson EC, Herd RM, Arthur PF, Wright J, Xu G, Dibley K, Oddy VH (1996) Possible physiological indicators for net feed conversion efficiency in beef cattle. *Proceedings of the Australian Society of Animal Production* **21**, 103–106.

Savietto D, Berry DP, Friggens NC (2014) Towards an improved estimation of the biological components of residual feed intake in growing cattle. *Journal of Animal Science* **92**, 467–476. doi:10.2527/jas.2013-6894

Schenkel FS, Miller SP, Wilton JW (2004) Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. *Canadian Journal of Animal Science* **84**, 177–185. doi:10.4141/A03-085

Shabat SKB, Sasson G, Doron-Faigenboim A, Durman T, Yaacoby S, Berg Miller ME, White BA, Shterzer N, Mizrahi I (2016) Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. *The ISME Journal* **10**, 2958–2972. doi:10.1038/ismej.2016.62

Shi W, Moon CD, Leahy SC, Kang D, Froula J, Kittelmann S, Fan C, Deutsch S, Gagic D, Seedorf H, Kelly WJ, Atua R, Sang C, Soni P, Li D, Pinares-Patiño CS, McEwan JC, Janssen PH, Chen F, Visel A, Wang Z, Attwood GT, Rubin EM (2014) Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. *Genome Research* **24**, 1517–1525. doi:10.1101/gr.168245.113

Tsilimigras MCB, Fodor AA (2016) Compositional data analysis of the microbiome: fundamentals, tools, and challenges. *Annals of Epidemiology* **26**, 330–335. doi:10.1016/j.annepidem.2016.03.002

Ungerfeld EM (2020) Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions. *Frontiers in Microbiology* **11**, 589. doi:10.3389/fmicb.2020.00589

Weimer PJ, Stevenson DM, Mantovani HC, Man SLC (2010) Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. *Journal of Dairy Science* **93**, 5902–5912. doi:10.3168/jds.2010-3500

Woloszynek S, Mell JC, Zhao Z, Simpson G, O'Connor MP, Rosen GL (2019) Exploring thematic structure and predicted functionality of 16S rRNA amplicon data. *PLoS One* **14**, e0219235. doi:10.1371/journal.pone.0219235

Yuste S, Amanzougarene Z, de Vega A, Fondevila M, Blanco M, Casasús I (2020) Effect of preweaning diet on performance, blood metabolites and rumen fermentation around weaning in calves of two beef breeds. *Animal Production Science* **60**, 1018–1027. doi:10.1071/AN19152

Handling editor: Di Mayberry