The potential of using rumen microbial gene abundances to improve feed efficiency in beef cattle

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Summary

The main aim of this study was to elucidate whether rumen microbial gene abundances (RMGA) can be used to predict residual feed intake (RFI), feed conversion ratio (FCR) and its component traits: daily feed intake (DFI), average daily gain (ADG), and thus RMGA could be applied as indirect trait for breeding of feed efficiency in beef cattle. RMGA were generated by whole metagenomic sequencing of rumen microbial DNA samples from 42 beef cattle, with extreme low and high FCR, selected from two feed efficiency trials. The results of the PLS analysis indicated that RMGA showed substantial potential to be used as predictors for RFI, FCR and its components DFI and ADG explaining 55 to 73% of their variation. While only 12 and 23 microbial genes were significantly associated with RFI and DFI, respectively, there were 166 and 167 affecting ADG and FCR. RFI and DFI were influenced partly by the same microbial genes and combined in the same microbial network clusters as was also the case for FCR and ADG. The results elucidate the likely potential of RMGA to predict the difficult and costly to measure trait feed efficiency, but have to be confirmed under the more challenging conditions of practical breeding programmes.

Keywords: feed efficiency, growth, microbiome, metagenomics, genetic improvement

Introduction

Feed costs typically account for 70 to 80% of the variable costs of beef production in the UK. Consequently, genetic improvement of feed efficiency is a key breeding objective due to its large economic and positive environmental impact on beef cattle production. However, measuring individual animal feed intake (e.g. using electronic feeders) on a sufficient number of animals to obtain accurate breeding values is very expensive and time consuming (Culbertson *et al*., 2015). Therefore, proxy methods are of substantial interest to predict feed intake or even feed efficiency on a large number of animals and in a shorter timeframe. Recently, Roehe *et al*. (2016) suggested that rumen microbial gene abundances (RMGA) may be able to predict feed efficiency of beef cattle. However, that study had only a few observations available, which were selected for high and low methane emissions, and did not analyse the components traits of feed conversion ratio (FCR), daily feed intake (DFI) or average daily gain (ADG). Another feed efficiency criterion applied in beef breeding is

residual feed intake (RFI), which may also be associated with RMGA. Therefore, the aim of this research was to identify whether RMGA are associated with FCR and its component traits, as well as RFI. A further objective was to identify whether microbial genes associated with feed efficiency, form clusters within the context of correlation networks.

Material and methods

Data of 42 animals were from experiments conducted at SRUC's Beef and Sheep Research Centre and selected for low and high FCR from two feed efficiency trials. The selection groups were balanced for all levels of experimental factors. Experimental factors were breed and diet, with the primary aim to investigate the effect of feed additives (nitrate or dietary lipid) on methane emissions, feed efficiency, growth, as well as carcass and meat quality in different breeds. Further descriptions of the experimental data are in Troy *et al*. (2015) and Duthie *et al*. (2016, 2017).

Traits

After an 8 week adaptation period to the experimental diets, the traits related to feed efficiency were measured on the steers over a 56 day test period. Individual feed intake was recorded using electronic feeders and calculated based on dry matter analysis of the diet as dry matter intake (DMI). Animals were weighed weekly and the linear regression of body weight on test date was used to obtain ADG (kg gain/day). FCR was calculated as ratio of DFI (kg DMI/day) and ADG (kg gain/day). RFI was obtained as deviation of actual DMI (kg/day) from the predicted DMI, estimated based on the linear regression of actual DMI on ADG, mid metabolic body weight and ultrasonic fat depth scanned at the end of the test period.

Metagenomic analysis

Animals were slaughtered in a commercial abattoir, where two rumen digesta samples were taken immediately after the rumen was opened to be drained. Metagenomic DNA was extracted from the rumen digesta samples following the protocol presented by Rooke *et al*. (2014). Metagenomic DNA was sequenced on Illumina HiSeq 4000 to obtain at least 45 million paired-end reads (up to 2 × 150 bp) per sample. The metagenomic reads were aligned to the KEGG genes database to identify the microbial gene abundances (Wallace *et al*., 2015). Only microbial genes with a relative abundance > 0.001% were included in the analyses.

Analysis

Partial least squares analysis (PLS, SAS Institute Inc., Cary, NC, USA) was performed to identify the most important microbial genes associated with feed efficiency and its component traits. Model selection was based on the 'variable importance for projection' (VIP) criterion, whereby microbial genes with a VIP < 0.8 contribute little to the prediction and are considered to be non-significant (Wold, 1995). Distinct functional clusters of microbial genes were identified using the network analysis software Miru (Kajeka Ltd., Edinburgh, UK). These networks consist of nodes representing microbial genes and the connecting edges determining the correlations between these genes.

Results

To obtain highly informative data in our expensive study, due to applying whole metagenomic sequencing, the power of the analysis was increased by selecting animals extreme in FCR. The means of FCR as well as RFI of the extreme groups were highly significantly different (Table 1). Interestingly, mean DFI and ADG were not significantly different between selection groups indicating that these component traits of FCR were not influenced by the selection.

Table 1. Trait differences between selected animals for low and high feed conversion ratio.

		FCR				
		Low		High		
Trait[1]	Unit	Mean	SD	Mean	SD	SE_{Diff}
DFI	kg/day	11.559[a]	1.279	11.919[a]	1.968	0.362
ADG	kg/day	1.653[a]	0.389	1.780[a]	0.430	0.089
FCR		6.626[a]	0.741	8.871[b]	1.207	0.218
RFI		-0.328[a]	0.682	0.431[b]	1.025	0.190

[1] DFI = daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; RFI = residual feed intake
[2] Means with different superscripts within each row are significantly different (P < 0.01)

Microbial genes to predict efficiency traits

The PLS analysis indicated that the number of microbial genes significantly associated with efficiency traits were largely different between ADG or FCR and DFI or RFI (Table 2). The small number of microbial genes affecting DFI and RFI may be due to the fact that these traits are more related to the animal's metabolism. For example, RFI is expected to be highly associated with protein turnover in animal cells, which seems to be affected only by a few microbial genes. Of the microbial genes, 88 affected both ADG and FCR and 9 both DFI and RFI. There were no further microbial genes commonly affecting the traits, suggesting that ADG and FCR have a common microbial gene association background as well as RFI and DFI. Interestingly, the few microbial genes associated with DFI and RFI explained more variation in those traits than the many genes associated with ADG and FCR. The microbial genes associated with DFI and RFI may have specific capabilities to provide, e.g. a cross-talk to epithelia cells to regulate the amount of feed intake or the protein turnover in animal cells.

Table 2. Partial Least Squares (PLS) analysis to predict traits associated with feed efficiency.

	Variation explained in:		Number of:
Trait[1]	Model factors (%)	Trait (%)	Microbial genes
DFI	50	72	23
ADG	64	55	166
FCR	61	68	167
RFI	40	73	12

[1] DFI = daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; RFI = residual feed intake

Network clusters of microbial genes

Network clusters of all microbial genes were generated based only on their relative abundances across animals and the clusters are highlighted containing microbial genes found by PLS analysis to be significant for prediction of the efficiency traits (Figure 1). More than 55% of the microbial genes associated with FCR and ADG were in cluster 6. For both DFI and RFI the main cluster was 4, containing more than 60 and 33%, respectively, of the microbial genes associated with those traits. These results reveal again that ADG and FCR were influenced by similar networks of microbial genes, as was also the case for DFI and RFI.

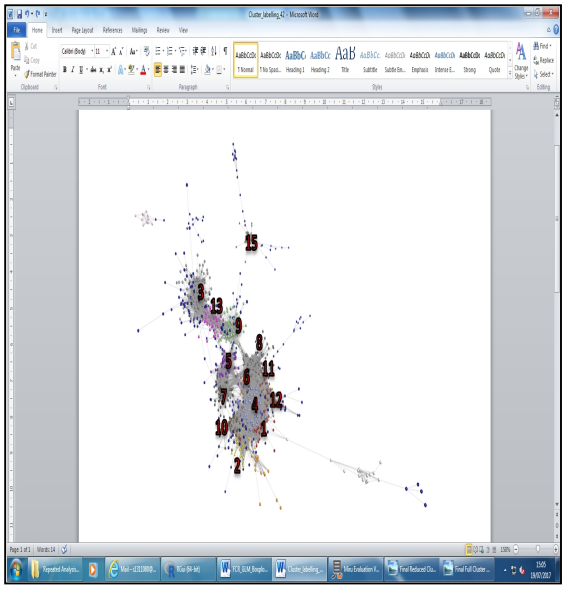

Figure 1. Network of microbial genes and their clusters of strongly correlated genes, in which each dot represents one microbial gene and short edges illustrate strong correlations.

Discussion

The results of the PLS analyses indicate that FCR ratio can be predicted by RMGA which confirms the results of Roehe *et al*. (2016), which were based on a substantially smaller sample size. In this study, we could additionally reveal that the component traits of FCR, DFI and ADG, as well as RFI can also be predicted by RMGA. This would allow a more general application of the proxy trait within breeding programmes using an index of the component traits of FCR or RFI (Kennedy *et al*., 1993; Wang *et al*., 2012). Some advantages of using the RMGA as an indirect trait to improve feed efficiency are that it can be obtained from a large number of live animals using a stomach tube and from slaughtered animals in the abattoir, the relative abundances of most identified microbial genes were normally distributed, and the function of many of the microbial genes are known. Even though the potential of using RMGA for improvement of feed efficiency is high, before its application, there have to be studies using practical data from breeding programmes. One challenge is the impact of the diet (which was known exactly in our study) but has to be approximated in the practical breeding programmes using, e.g. herd-year season effect. Another challenge will be to develop cost-effective methods for rumen sample collection, DNA extraction and the

determination of the abundances of the microbial genes using, e.g. a newly developed functional microbial gene array. But generally, many opportunities are opened up for breeding using rumen microbial information not only for feed efficiency but also for traits such as methane emissions, meat quality (Omega-3 fatty acids) and animal health.

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