ACROSS-BREEDS SYSTEMS BIOLOGY ANALYSIS REVEALS KEY GENES CONTRIBUTING TO FEED EFFICIENCY IN BEEF CATTLE

K. Keogh[1,2], D.A. Kenny[2], P.A. Alexandre[1], M. McGee[3] and A. Reverter[1]

[1]CSIRO Agriculture & Food, Queensland Bioscience Precinct, 306 Carmody Rd., St. Lucia, Brisbane, QLD 4067, Australia
[2]Animal and Bioscience Research Department, Teagasc, Grange, Dunsany, Co. Meath, Ireland
[3]Livestock Systems Research Department, Teagasc, Grange, Dunsany, Co. Meath, Ireland

SUMMARY

Provision of feed in beef production systems is a major determinant of overall profitability as it typically accounts for over 75% of the variable cost. Thus, improving cattle feed efficiency by way of determining the underlying molecular control and subsequently selecting for feed efficient cattle through genomic selection provides a method through which feed costs may be reduced. The objective of this study was to undertake gene co-expression network analysis on RNAseq data generated from *Longissimus dorsi* tissue samples collected from steers divergent for residual feed intake (RFI) within two contrasting breed types (Charolais and Holstein-Friesian). Several gene categories, including differentially expressed genes (DEG) based on the contrasts of both breed and RFI phenotype as well as key transcription factors and proteins secreted in plasma were utilised as nodes of the gene co-expression networks. Significant network connections were identified using an algorithm that exploits the dual concepts of partial correlation and information theory (PCIT). Results revealed 530 and 531 DEG for the RFI and breed contrasts, respectively. PCIT network analysis resulted in the formation of one RFI specific cluster which included genes related to metabolic processes and cell cycle. A second cluster which included genes related to both RFI and breed was enriched for immune-related pathways such as coagulation system and the complement cascade. This latter network was of particular interest due to the potential identification of genes contributing to RFI that are sufficiently robust across breed type. Moreover, genes included within this network also encode proteins secreted in plasma, highlighting the potential use of these genes as blood-based biomarkers for RFI in beef cattle.

INTRODUCTION

Within beef production systems, provision of feed is a major determinant of overall profitability, as it accounts for up to 75% of the total variable costs of production (Kenny *et al*. 2018). Consequently, research related to the identification and subsequent breeding of beef cattle with improved feed efficiency has received attention to alleviate the high input costs and environmental footprint associated with beef production. In particular, residual feed intake (RFI), defined as the difference between an animal's actual and predicted feed intake, has become the feed efficiency measure of choice due to its independence of both growth and body size (Fitzsimons *et al*. 2017). However, despite research efforts aimed at uncovering the molecular control of RFI in cattle, genes which are robust across varying breed type contributing to RFI are yet to be identified (Kenny *et al*. 2018). This is undoubtedly due to the multifaceted nature of the RFI trait as well as the varying experimental parameters employed across different studies, such as breed types, dietary sources and stage of development evaluated, ultimately confounding the subsequent outcome.

Thus, the objective of this study was to undertake gene co-expression network analysis on *Longissimus dorsi* (LD) transcriptomic data collected from steers divergent for RFI within two contrasting breed types (Charolais and Holstein-Friesian). Specifically, differentially expressed genes (DEGs) for both RFI and breed contrasts were used as nodes for the co-expression network analysis. The LD muscle was chosen as a target tissue due to its' economic importance, in addition

to its responsiveness to variation in RFI in cattle (Fitzsimons *et al*. 2017).

MATERIALS AND METHODS

All procedures involving animals in this study were reviewed and approved by the Teagasc Animal Ethics Committee and were conducted under an experimental licence issued by the Irish Health Products Regulatory Authority (AE19132/P029).

This experiment was conducted in Ireland under moderate non-extreme climatic conditions as part of a larger research programme designed to examine the within-animal repeatability of intake, growth, and feed efficiency between varying stages of development in Charolais and Holstein-Friesian beef steers (Coyle *et al*. 2016). In total, 167 steers (90 Charolais and 77 Holstein-Friesian) were sourced from commercial farms in Ireland, parentage was included within the animal selection process so as to avoid selecting genetically related animals. At the start of the trial Charolais and Holstein-Friesian steers were on average 283 and 307 days of age, respectively. Following a dietary adaptation period, dry matter intake (DMI) and growth rate were measured over a 70-day feeding trial, during which all steers were offered the same high-energy diet consisting of *ad libitum* concentrates plus a restricted allowance of grass silage daily. Throughout the trial all steers were accommodated indoors, utilising a Calan gate feeding system. The residuals of the regression of DMI on average daily gain (ADG), and mid-test metabolic body weight within each breed were used to compute individual RFI coefficients for each steer (GLM procedure of SAS9.3). Residual feed intake was calculated for each animal as the difference between actual and predicted DMI. Within each breed, steers were ranked for RFI, with high-RFI (feed-inefficient; n=12) and low-RFI (feed-efficient; n=12) steers selected for each breed separately. Samples of LD tissue were collected through punch biopsy from all high-RFI and low-RFI steers following completion of the 70-day dietary trial. Tissue samples were washed with sterile DPBS and immediately snap frozen in liquid nitrogen before subsequent storage at -80°C.

Total RNA was purified from all tissue samples using the Qiagen RNeasy Universal kit (QIAGEN, UK), according to the manufactures instructions as previously described (Higgins *et al*. 2019). The quality of the resultant RNA was assessed using the RNA 6000 RNA Nano Lab Chip Kit and the Agilent Bioanalyser 2100 (Agilent Technologies Ireland Ltd., Dublin, Ireland). All samples passed quality control with RNA integrity numbers greater than 8. The Illumina TruSeq sample preparation kit (Illumina, San Diego, CA) was utilized to construct cDNA libraries for each sample, following which cDNA libraries were sequenced using the Illumina HiSeq 2500 sequencing platform (Illumina, San Diego, CA). Bioinformatic analysis was undertaken as previously described in Higgins *et al*. (2019) including the removal of sequencing adapters and low quality reads using cutadapt (v. 1.13) and quality control of sequencing reads undertaken using FastQC (v. 0.11.5). Trimmed sequencing reads were mapped to the bovine reference genome (ARS-UCD1.2) and also quantified using STAR (v.2.5.1). Differentially expressed genes were detected between each of the two main contrasts: (i) High-RFI versus Low-RFI; and (ii) Charolais versus Holstein-Friesian) using the Bioconductor package, EdgeR (v3.20.9). Gene expression was estimated as Counts Per Million (CPM) and genes were retained for subsequent analysis only when presented in at least 1 CPM in at least half of the samples for each contrast. The top 5% most significant genes (based on Benjamini-Hochberg corrected P-value of differential expression) in each contrast were considered DEG and were selected for subsequent inclusion in the co-expression network analysis. Additionally key transcription factors (TF) and proteins secreted in plasma were also utilised as nodes within the gene co-expression networks. For gene co-expression network analysis, genes selected based on differential expression, as key TF and secreted in plasma were used as nodes and significant edges between nodes identified using the Partial Correlation and Information Theory (PCIT) algorithm (Reverter and Chan 2008). The output of PCIT was then visualised using Cytoscape (V3.9.1)

(Shannon *et al*. 2003) including only significant correlations above 0.9 and their respective genes. Functional enrichment of gene networks was performed using Ingenuity Pathway Analysis (IPA).

RESULTS AND DISCUSSION

A significant difference (P<0.0001) in RFI value was evident for each breed (Charolais: Low-RFI=-0.53, High-RFI=0.55; Holstein-Friesian: Low-RFI=-0.64, High-RFI=0.7). For the RFI and breed contrasts, 530 and 531 DEGs were identified, respectively. Of these 114 genes (12.4%) were common between both contrasts. A total of 1,061 DEG, 292 TF and 405 genes encoding proteins secreted in plasma were identified as associated with variation in both RFI and breed type. Gene co-expression network visualisation of significant correlations between genes above 0.9 equated to 298 genes with 5,625 connections, the main clusters of interest are presented in Figure 1.

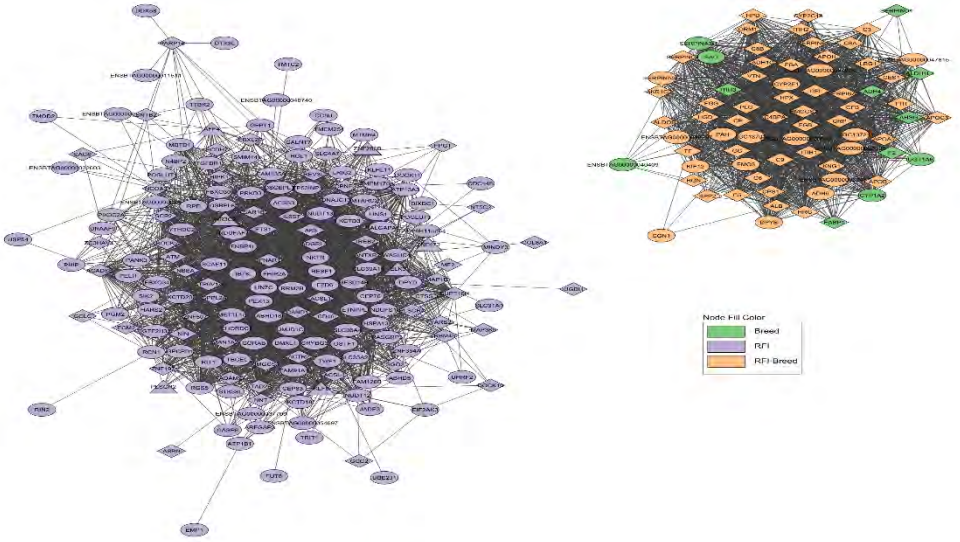

Figure 1. Gene co-expression network of genes related to RFI in Charolais and Holstein-Friesian steers. Node colour are relative to the differential expression contrast: purple represents RFI only; green represents breed only and orange represents genes differentially expressed in both RFI and breed contrasts. Genes encoding transcription factors and proteins secreted in plasma are represented as triangle and diamond shapes, respectively

Network visualisation highlighted a clear cluster of genes specifically related to RFI (purple), whilst a second cluster depicted genes related to both RFI and breed contrasts (RFI-Breed, orange). Functional analysis of the RFI specific cluster of co-expressed genes highlighted pathways related to mitochondrial fatty acid oxidation including fatty acid β-oxidation (adj.P<0.005) and mitochondrial L-carnitine shuttle pathway (adj.P<0.01), suggesting a role for mitochondrial fatty acid oxidation towards variation in RFI in beef cattle. Processes related to fatty acid oxidation have previously been implicated towards divergence in RFI in varying tissue types (subcutaneous adipose: McKenna *et al*. 2018: liver: Taiwo *et al*. 2022), with up-regulation of fatty acid oxidative processes within the feed efficient (low-RFI) cattle apparent in each study. Indeed, McKenna *et al*. (2018) postulated that the increased expression of fatty acid oxidative genes in the low-RFI animals may be due to the efficient cattle directing metabolic processes towards alternative substrate partitioning and fatty acid breakdown in order to facilitate their lower dietary intake.

A potential role for immune processes towards variation in RFI has been established across varying experimental designs (Fitzsimons *et al*. 2017; Kenny *et al*. 2018); however, specific immune related processes are conflicting across experimental designs. Pathway analysis of the network of co-expressed genes related to both RFI and breed revealed an enrichment of immune-related processes including coagulation system and complement cascade (P<0.001). Moreover, genes included within this network and pertaining to coagulation (*FGA*, *FGB* and *FGG*) and complement system (*C3*, *C5*, *C9*, *CFH*, *CFI* and *CRP*) pathways were previously reported as differentially expressed between cattle divergent for RFI across various breed types including Angus, Nellore, Holstein-Friesian and Charolais (Chen *et al*. 2011; Tizioto *et al*. 2016; Weber *et al*. 2016; Higgins *et al*. 2019). Moreover, the aforementioned genes also encode proteins secreted into plasma, suggesting a potential role for these genes as blood-based biomarkers for RFI in beef cattle.

CONCLUSION

Results from this study provide potential candidate genes, pathways and networks related to feed efficiency in beef cattle. The RFI-breed network is of particular interest for the potential identification of robust genes contributing to the RFI trait irrespective of breed type. Moreover, genes included within this network were also genes coding proteins secreted in plasma, highlighting the genes potential to be explored as blood-based biomarkers for the RFI trait in beef cattle. However, extensive functional experimental validation for the candidate genes and pathways identified in this study is warranted.

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