Gene networks contributing to compensatory growth in hepatic tissue in cattle

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Summary

Compensatory growth is an accelerated growth phenomenon observed in animals upon re-alimentation following a period of dietary restriction and is utilised worldwide in animal production systems as a method to lower feed costs. Gene co-expression network analysis was performed on the hepatic transcriptome of cattle that had undergone compensatory growth. The relationship between modules of co-expressed genes and traits contributing to compensatory growth were assessed. A module of co-expressed genes was identified as positively correlated with dry matter intake ($r = 0.77$, $P = 0.01$). Functional annotation of genes within this module revealed gene ontology terms including RNA splicing and cell division. Our results suggest that compensatory growth of hepatic tissue in cattle may be the consequence of the expression of genes involved in these biological processes. Additionally, hub genes in this module may represent potential biomarkers for the selection of cattle with enhanced compensatory growth potential.

Keywords: gene networks, compensatory growth, cattle

Introduction

Feed-related costs account for up to 75% of the variable costs associated with beef cattle production (Finneran *et al*., 2010), thus any method through which these costs may be reduced would be of benefit to the beef industry. The compensatory growth phenomenon is commonly utilised to provide such a reduction through a reduction in overwintering feed costs (Keogh *et al*., 2015). Compensatory growth is an accelerated growth displayed upon re-alimentation following a prior dietary restriction (Hornick *et al*., 2000). However, although widely utilised, the biochemical mechanisms controlling this phenomenon are yet to be elucidated fully. To date reports in the literature on the molecular control regulating the expression of compensatory growth in cattle have focused on the identification of differentially expressed genes (Connor *et al*., 2010; Keogh *et al* 2016a,b, 2017). However, such studies do not explain the interactions between genes governing the expression of a trait. Identification of highly co-expressed genes that contribute to the expression of complex traits may reveal more information on the molecular control of a particular trait. Moreover, the identification of hub genes, which are important in regulating the expression of several other genes within a network of co-expressed genes, may hold potential as biomarkers for the selection of a trait of interest. The objective of the current study was to identify modules of co-expressed genes significantly associated with the expression of compensatory growth in hepatic tissue through gene co-expression network analysis. The liver was chosen as a target tissue as it is a central metabolic organ that is responsive to both dietary restriction and subsequent re-alimentation induced compensatory growth (Keogh *et al*., 2015).

Material and methods

This experiment was conducted as part of a research programme designed to examine the physiological control of compensatory growth in growing beef cattle (Keogh *et al*., 2015). Purebred Holstein Friesian bulls (n = 30; mean live-weight 370 ± 35 kg; mean age 479 ± 15 d) were blocked on the basis of live-weight and age and assigned within block to one of two dietary regimens: (i) restricted feed allowance for 125 days followed by *ad libitum* access to feed for a further 55 days (RES; n=15) or (ii) *ad libitum* access to feed throughout the trial (ADLIB; n=15). The first 125 days of the trial were denoted as Period 1 and the subsequent 55 days, Period 2. All animals were offered a 70:30 concentrate: forage (grass silage) diet throughout the trial. Target growth rates for RES and ADLIB were 0.6 kg day^{-1} and in excess of 1.5 kg day^{-1} during Period 1, respectively. Diets were fed individually, with the proportion of feed required, based on each animal's own individual bodyweight and all animals were weighed regularly throughout the trial. Following completion of Period 2 all animals were slaughtered in an EU licensed abattoir, hepatic tissue samples were collected from all animals. Tissue samples were washed with sterile DPBS and immediately snap frozen in liquid nitrogen before subsequent storage at -80°C.

RNA isolation, cDNA library preparation, RNAsequencing and bioinformatics analysis are described in Keogh *et al*. (2016a). The weighted gene co-expression network analysis (WGCNA) software package (Langfelder and Horvath, 2008) was used to identify modules of co-expressed genes, which were then correlated with traits associated with the expression of compensatory growth. Traits included average daily gain (ADG), feed conversion ratio (FCR) and dry matter intake (DMI). RNAseq read count data were filtered for lowly expressed genes and normalised in EdgeR by read counts per million mapped reads, (adjusted for sample library size). Normalised count data were then $Log_2(x+1)$ transformed in R. Networks of co-expressed genes were constructed using WGCNA within R. Unsigned, weighted correlation network construction and module detection was performed using the automatic one-step function, blockwise Modules. The resulting modules of co-expressed genes were assigned colour names by the software. Relationships between modules of co-expressed genes and trait data were then calculated by Pearson correlation. Modules with statistically significant ($P < 0.05$) correlations were selected for further analysis as potentially biologically interesting modules associated with the expression of compensatory growth in hepatic tissue. Gene ontology analysis was then performed on genes from each module identified as significantly correlated with trait data. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used for functional annotation of co-expressed genes within modules. Gene ontology terms were considered significant if the adjusted p-value was less than 0.05. The top hub genes of significant co-expressed modules were then visualized using Cytoscape software.

Results and discussion

Following a prior period of dietary restriction, cattle that underwent compensatory growth displayed accelerated growth, growing at 1.8 times the rate of their non-dietary restricted counterparts during Period 2 (Keogh *et al*., 2015). WGCNA identified 11 modules of co-expressed genes, 3 of which were significantly correlated with trait data. The turquoise module was positively correlated with ADG ($r = 0.69$, $P = 0.03$), the green module was negatively

correlated with ADG (r = -0.7, P = 0.02), and the red module was positively correlated with DMI (r = 0.77, P = 0.01). As the red module of co-expressed genes displayed the strongest correlation and was also the most significantly associated with the traits evaluated, it was further investigated in terms of its contribution to compensatory growth in cattle. Functional annotation of co-expressed genes in this module revealed biological processes involved in gene expression, and cellular division (Table 1).

Table 1. Functional annotation of red module of co-expressed genes significantly associated with DMI during compensatory growth.

Gene Ontology term in red module	Ontology	P value
mRNA processing	Biological process	2.20E-01
RNA splicing	Biological process	2.20E-01
Protein ubiquitination	Biological process	2.70E-01
Cell division	Biological process	4.80E-01
RNA secondary structure unwinding	Biological process	4.40E-01

Further evaluation of the top hub genes (Figure 1) within the red module revealed genes that code for proteins involved in metabolism (*ADSL*, *PFKFB1*), gene expression (*CDC40*, *MAFG*, *TLE1*, *WRAP53*) and growth regulation (*DDIT4*, *KDR*, *MXD1*, *TOB1*).

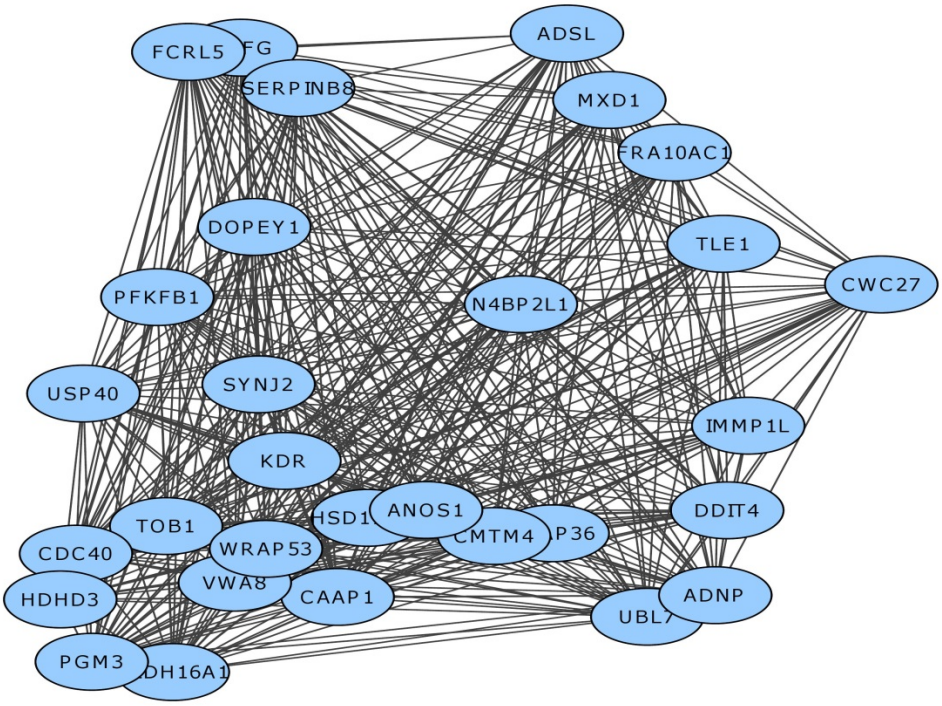

Figure 1 Network of co-expressed genes significantly associated with DMI in animals undergoing compensatory growth.

Top hub genes (Figure 1) included those that have previously been described in relation to the expression of compensatory growth including *IMMP1L* (Connor *et al*., 2010), *KDR* and *MXD1* (Keogh *et al*., 2016b), *PFKFB1* (Connor *et al*., 2016a), and *USP40* (Keogh *et al*., 2016b). Interestingly, the top genes in the current hepatic gene co-expression study within the

significant module were not identified as differentially expressed in the differential gene expression RNAseq study of the same samples used in this study (Keogh *et al*., 2016a), further establishing the utility of gene co-expression analyses and importance of knowledge of gene interactions in terms of identifying genes important to particular traits of interest. Greater DMI has been reported during compensatory growth (Sainz *et al*., 1995; Keogh *et al*., 2015), with an increased appetite thought to be contributing to the expression of compensatory growth in cattle (Keogh *et al*., 2015). Additionally the liver has been shown to be one of the most responsive tissues to dietary restriction and also subsequent re-alimentation (Keogh *et al*., 2015), with fluctuations in the weight of the organ directly proportional to dietary intake (Johnson *et al*., 1990). Our results suggest that compensatory growth of hepatic tissue in cattle may be the consequence of a change in feed intake, which is not unexpected given the metabolic role of the liver as well as its direct response to feeding level (Johnston *et al*., 1990), with genes involved in gene expression and growth potentially contributing to overall body compensatory growth. Additionally, hub genes identified in the red module may represent potential biomarkers for the selection of compensatory growth in cattle.

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