RNA-SEQUENCING OF MUSCLE FROM PIGS DIVERGENT IN RESIDUAL FEED INTAKE AND INTRAMUSCULAR FAT CONTENT

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Abstract — Residual feed intake (RFI) is the difference between actual feed intake and predicted feed requirements and RFI status has an influence on aspects of meat quality. Our objective was to investigate the molecular mechanisms underpinning the relationship between RFI and meat quality in porcine muscle. Composition (n=40) and RNA-Seq (n=16) analysis were carried out in *Longissimus thoracis et lumborum* of pigs differing in RFI status. 413 genes were differentially expressed. Significant canonical pathways represented among the differentially expressed genes included FXR/RXR & LXR/RXR activation and acute phase response signalling. The findings suggest that more efficient pigs might have increased absorption of lipids in muscle tissue which could contribute to growth processes and differences in intramuscular fat accumulation.

Key Words – feed efficiency, meat quality, gene expression, transcriptomics

I. INTRODUCTION

Residual feed intake (RFI) is the difference between actual feed intake and predicted feed requirements for maintenance and growth [1]. More efficient (low RFI) pigs tend to be leaner and have been shown to display impaired meat quality characteristics [1,2]. The molecular mechanisms contributing to differences in RFI and their relationship with meat quality are not fully elucidated, therefore the objective of this study was to profile the *Longissimus thoracis et lumborum* (LTL) muscle transcriptome and identify differentially expressed (DE) genes, functions and canonical pathways associated with RFI and meat quality.

II. MATERIALS AND METHODS

This study involved 40 Maxgro (Hermitage Genetics) x (Landrace x Large White) pigs from low and high RFI groups, selected out of a larger group of 138. *Longissimus thoracis et lumborum* (LTL) tissue was collected from each carcass at slaughter. For meat quality, samples were trimmed of external fat, homogenized and intramuscular fat content (IMF) was measured with NMR Smart Trac & Smart 5 Rapid Fat Analyser (CEM Corporation, USA) using AOAC method 985.14, 1990. For transcriptomic profiling, snap frozen muscle samples (8 low RFI and 8 high RFI) were ground into fine powder in liquid nitrogen. Total RNA was isolated using Tri-Reagent (Sigma-Alrich, Taufkirchen, Germany). RNA library preparation was carried out using the TruSeq Stranded mRNA protocol. Following RNA sequencing with Illumina HiSeq2500, high quality paired-end reads were mapped to the reference [3] using TopHat (2.1.0). Count reads were assigned to the gene features using the HTSeq (0.6.1) [4] and differential gene expression analysis was performed using edgeR package (3.1.1, www.R-project.org). In order to validate the RNA-seq results, four DE genes were selected for quantitative real-time PCR (qPCR), which was carried out on the LightCycler 96 system (Roche Diagnostics, Mannheim, Germany). Gene symbols for DE genes, related fold changes and corresponding P-values were submitted to Ingenuity Pathway Analysis (IPA; Ingenuity® Systems, www.ingenuity.com). Benjamini-Hochberg corrected P-values were used to detect significantly enriched bio-functions and canonical pathways.

III. RESULTS AND DISCUSSION

Intramuscular fat content was 1.89% in high and 1.49% in low RFI pigs and, although differing ~~in~~ by a small amount, this was significant ($P < 0.05$). We have previously shown that these pigs differed in other meat quality traits, including pH and water holding capacity [2].

An average of 104.4 million high quality paired-end reads per sample were mapped to the reference with a mean of 80.9% mapping efficiency. A total of 413 genes were differentially expressed ($P < 0.05$), with 225 at least 1.5-fold

differentially expressed in low versus high RFI pigs. The most altered genes were *CRP* (fold change = 44.6; low RFI > high RFI) and *ACTG2* (fold change = -2.37). Correlation between RNA-seq and qPCR data was significant for all four examined transcripts and ranged from 0.90 to 0.93. Functional enrichment analysis revealed 44 functions and 11 canonical pathways significantly enriched for DE genes ($p < 0.001$). The three most significant functions and pathways altered in RFI groups are presented in Table 1.

Table 1 Most significantly enriched biological functions and canonical pathways in RFI groups.

Biological Function	B-H P-value*	Canonical Pathway	-log(p-value)	z-score
Cellular Movement	3.27E-22 - 2.39E-06	FXR/RXR Activation	16.7	NA
Hematological System Development and Function	3.52E-22 - 2.55E-06	LXR/RXR Activation	14.9	3.27[£]
Immune Cell Trafficking	3.52E-22 - 2.39E-06	Acute Phase Response Signalling	14.7	1.60

*Range of B-H multiple testing correction P-values of enriched biological functions within the category; NA: No activity pattern available;
[£]Significantly activated (z-score > 2) pathways in low RFI pigs.

Functions affiliated with recruitment of leucocytes to infected or injured tissue including movement, migration, adhesion and homing were significantly increased in low RFI pigs. Acute phase response, a rapid inflammatory response that can be triggered by infection and tissue injury [5], was also significant with a direction towards activation in the more efficient pigs. Muscle of low RFI pig may thus be characterised by faster recovery and shorter duration of infection. Activation of Liver X receptor (LXR) pathway and enrichment of DE genes in Farnesoid X receptor (FXR) pathway which have function in transport of cholesterol [6] and bile acid synthesis [7] respectively, might indicate that low RFI pigs have enhanced muscular absorption of lipids and also increased fatty acid oxidation [8], which might relate to reduced intramuscular fat level [8] in agreement with phenotypical observations of low RFI pigs.

IV. CONCLUSION

These findings suggest that more efficient pigs might exhibit increased muscular absorption of lipids as well as enhanced lipid delivery to the cells, which could then be utilized for maintenance and growth. Moreover, a shorter duration of potential infection in the low RFI pigs could further conserve energy for maintenance and growth.

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