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Interaction of gene expression pathways, breed and diet on the nutritive and flavour aspects of pigmeat



Key external stakeholders:

Pig producers and pigmeat processors

Practical implications for stakeholders:

The outcome of this research provides more in-depth understanding of factors such as breed, muscle, sex and diet which can have significant effect on meat quality, in particular intramuscular fat (IMF) levels.

- A number of genetic pathways which respond to these factors through alterations in their expression levels have been identified.
- Blood parameters provide potential as novel routine markers for quality characteristics with circulating triglyceride and albumin levels associated with dietary treatments.
- Many of the genes identified as differentially expressed between Duroc and Pietrain breeds are likely to harbour genetic variability in their regulatory regions that may ultimately have applications in meat management and/or genome-assisted animal selection programmes. This project shows the potential of nutrigenomics to optimise the efficacy of pork production regimes.

Main results:

- Generation of a knowledge baseline of quality and gene expression differences between two breeds (Duroc and Pietrain) with regard to IMF deposition.
- Demonstration, at a molecular level, that the degree of IMF deposition is as a result of a suite of diverse genomic responses with the importance of signaling pathways, lipid, fatty acid and steroid metabolism and the immune response highlighted.
- Highlighting that there is a muscle effect, in relation to IMF content, in the influence of restricted lysine treatment on meat quality with the *semimembranosus* (leg) muscle responding more strongly than the striploin muscle. Breed also influenced the response with Duroc muscle (both muscles) exhibiting a greater response to the restricted diet.

Opportunity / Benefit:

Information generated in the course of this project will aid the improvement of meat quality traits in Irish pork. The results highlight the importance of breeding and selection programs and the need to emphasise improvement in meat quality without compromising the production gains from traditional selection for lean carcass and high growth rate. The new knowledge generated about the Duroc breed is highly relevant as there is a gradual increase in the proportion of genetics of breeds such as Duroc in Irish and European commercial operations. This project may potentially open up the application of nutrigenomics to improve the efficacy of pork production regimes. The control and manipulation of these genes is a promising pathway of research for the future and Teagasc welcomes expressions of interest in this research.

Collaborating Institutions:

UCD

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1. Project background:

Palatability and technological quality are complex properties in pork, which are influenced by multiple interacting factors. These include breed, genotype, feeding, pre-slaughter handling, stunning, slaughter and hanging methods, chilling and storage conditions. Divergent breeding goals over time have led to variation in certain meat quality traits among specialised porcine breeds. Unraveling complexities in gene expression patterns in pork muscle that is divergent for meat quality traits variation, using gene expression technology, can expand our understanding of the genomic, and hence biological basis for this divergence. Two very different breeds such as Duroc and Pietrain provide widely divergent backgrounds in which to examine the range of genomic factors influencing the regulation of fat content in pork. Differences have been reported in meat quality measures of pH at 24h post-slaughter, marbling and water-holding capacity in these breeds, with Duroc being generally superior for most traits, apart from fatty acid profile. The dynamic response of the muscle genome to its environment is predicted to have an impact on the biology of muscle and on the ultimate quality of meat produced. We aimed to subject these divergent breeds to treatments which would significantly vary their meat quality and provide model systems for the exploration of genetic control of important meat quality traits. One of the primary influences on meat quality is the level and type of intramuscular fat. The aim of this study was to firstly provide a muscle resource that was divergent for quality and secondly to provide information on the genetic regulation of fat content in pigmeat through gene expression analysis of the divergent pigmeat.

2. Questions addressed by the project:

- Is it possible to identify pigmeat that is significantly divergent in important meat quality characteristics through sampling divergent breeds and dietary intervention regimes?
- What are the genes which are more altered in their activity (expression) in pig muscle with high intramuscular fat content compared with lower intramuscular fat content?
- Do divergent breeds differ in their meat quality and genomic response to the same experimental conditions?
- Do individual muscles differ in their response to the same experimental conditions?
- Which biological processes and genetic pathways are most important in the regulation of fat content in porcine muscle?

3. The experimental studies:

To achieve the aims of the study the contribution of gene expression in combination with factors such as genetic variation and environmental effects, were investigated. Two experiments were designed to produce muscle tissue that was significantly divergent in important aspects of meat quality. These experimental trials were each followed up with a gene expression study (using Affymetrix Porcine GeneChip® which represented about 22,000 genes) to identify which genes were most influential in regulating meat quality.

The project was conducted in two phases:

- Experiment 1 comprised a meat quality analysis and gene expression study of *longissimus* (striploin) muscle from Duroc and Pietrain background pigs reared under identical environmental and dietary conditions. This aimed to identify whether Pietrain-origin and Duroc-origin muscle were divergent for meat quality and to identify the gene expression differences across breeds with relevance to pigmeat quality, whilst controlling for all other influences on quality.
- Experiment 2 comprised a similar design. However, an experimental dietary treatment predicted to result in altered fat content was further overlaid on experimental Duroc and Pietrain samples. Both *longissimus* and *semimembranosus* (leg) muscle of Duroc and Pietrain breeds were sampled. Reducing levels of essential amino acids in the animal's diet has been reported to increase intramuscular fat levels in pork, therefore the trial was based on comparing meat quality and gene expression in muscle of pigs raised with normal and restricted protein. Studying the treatment effects in two very different breeds such as

Duroc and Pietrain added scope to the study by providing widely divergent backgrounds in which to see differences in intramuscular fat (IMF) level. Gene expression analysis focused on the *semimembranosus* muscle as composition analysis indicated larger differences in IMF content of this muscle in response to the experiment. A panel of divergent pork samples for each of the following traits was identified; tenderness, intramuscular fat, drip loss and PSE-like and DFD-like for each breed.

4. Main results:

- Experiment 1 showed that Pietrain and Duroc-origin loin muscle was significantly divergent in relation to meat quality, with Pietrain muscle displaying higher concentrations of polyunsaturated fatty acids (PUFA), polyunsaturated/saturated ratio, omega 6, omega 6/3 ratio and meat colour (redness). The *longissimus* muscle of the Duroc breed had higher IMF content and Pietrain *longissimus* muscle displayed a higher omega 3 percentage than Duroc animals.
- Through experiment 1 differential gene expression was determined between *longissimus* muscle of Duroc and Pietrain origin. Approximately 100 differently expressed genes were detected. The genes were associated with fatty acid metabolism, protein modification, protein binding and protein transport.
- Experiment 2 was mainly focused on IMF content, and results indicate that breed, muscle, and dietary intervention all had significant influence on intramuscular fat (IMF) levels.
- *Longissimus* muscle displayed an overall lower level of fat compared to *semimembranosus*.
- As hypothesized, *semimembranosus* muscle from animals offered a restricted diet displayed higher levels of IMF in both breeds.
- The experimental diet had a significant effect on longissimus IMF levels in Duroc animals but there was no difference between treatments in pork longissimus of Pietrain backgrounds.
- We conclude that in relation to meat quality, a) *semimembranosus* muscle responded to the restricted protein treatment more strongly than the *longissimus* muscle; b) muscle of Duroc origin had a greater response to the restricted protein treatment.
- Blood parameters were also investigated to determine if it was possible to correlate meat quality parameters with easily measured blood traits. Serum concentration of albumin was found to decline over the course of the trial and dietary restriction had a significant effect on circulating albumin.
- The GeneChip® experiments revealed a total of 454 and 372 genes were significantly different in their expression levels according to dietary treatment for Pietrain and Duroc females. For males, 433 and 264 genes were significantly differentially expressed according to dietary treatment for Pietrain and Duroc.
- The biological processes in which these genes are involved were diverse but included lipid, fatty acid and steroid metabolism and signal transduction. For example, numerous genes involved in leptin, PPARα and arachidonic acid signalling were notably perturbed in relation to IMF level. SCD, the critical catalyst of the conversion of saturated fatty acids to monounsaturated fatty acids (MUFA) was highly upregulated in higher fat tissue.
- The metabolic processes and pathways altered in response to differential accumulation of IMF offer insights on the mechanisms regulating IMF content in pig and potentially also on obesity phenotypes in the human population.

5. Opportunity/Benefit:

This research has led to a more in-depth understanding of the pathways and processes underpinning variability in pork quality and in particular provides information of relevance to the production of consistency in meat quality.

The results from both phases indicate that a number of factors such as breed, muscle, sex and diet can have significant effect on IMF levels. We identified a number of genetic pathways which respond to these factors through alterations in their expression levels. There is now a need to redirect breeding and selection programs to emphasise improvement in meat quality without compromising the production gains from traditional selection for lean carcass and high growth rate. In this regard the information is of benefit to the Irish meat industry (both processors and producers) and to the consumer.

In addition this research of benefit to the scientific community as platforms for assessing gene expression have been successfully applied to better describe the biochemical pathways and processes underpinning pork traits of economic relevance.

6. Dissemination:

The outputs from this research have been, and continue to be, disseminated to stakeholders (meat

processors, scientific community, government agencies) in a number of ways. Key findings of particular relevance to industry and government agencies are communicated through formal and informal methods, including RELAY workshops. A selection of publications is shown below.

Main publications:

Aslan, O., Hamill, R.M., Mullen, A.M., Davey, G., Gladney, C., Gil, M. and Sweeney, T. (2012). 'Promoter polymorphisms in a key cytoskeletal gene (Ankyrin 1) are associated with intramuscular fat and water-holding capacity in porcine muscle.' *Molecular Biology Reports*, 39 (4): 3903-3914

Pannier, L., Corcoran, D., Hamill, R., Sweeney, T. and Mullen, AM. (2008) 'Gene expression profile in two pig breeds using the Affymetrix GeneChip® Porcine Genome Array.' In Book of abstracts, *Agricultural Biotechnology for a competitive and sustainable future*, Cork, Ireland. p 4 24-27th August.

Mullen, A.M., Stapleton, P.C., Corcoran, D., Hamill, R.M. and White. A. (2006). 'Understanding meat quality through the application of genomic and proteomic approaches'. *Meat Science*, Volume 74, Issue 1, September 2006, Pages 3-16

Popular publications:

Hamill, R., Corcoran, D. and Mullen, A.M. Beefing up meat quality. *TResearch*. Volume 2, 2, p14-17 Summer 2007

Mullen, A.M., Corcoran, D., Hughes, L. and Hamill, R.M. (2007) Genomic and proteomic approaches to understanding and enhancing meat quality. FeedInfo.com. Available www.feedinfo.com

Hamill, R.M., Aslan, O., Mullen, A.M., O'Doherty, J.V., McBryan, J., Morris, D.G. and Sweeney, T. (2011). 'Transcriptomic profiles of porcine *M. semimembranosus* divergent in intra-muscular fat content'. *International Symposium on Animal Functional Genomics*, Dublin, October 10-12 2011.

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