Key Conclusion continued

However, genes or proteins related to the heat shock protein family, muscle fiber characteristics or metabolic enzymes may be potential markers of beef tenderness. Tended together, these results suggest there is now a requirement to adapt or modify genomic prediction tests for beef quality to specific rearing practices, bovine breed, animal type and beef cut. Following from this research, the private company IMAXIO now offers a bovine muscle transcriptomic analysis service on muscle samples from alive animals for academic or professional partners. The dot-blot quantitative tool for the analysis of several proteins in many muscle samples will be soon available for stakeholders as well.

Main scientific publications:


EAAF Publication 123 Wageningen Academic Publishers, Wageningen, The Netherlands, p. 81-82


Glossary

Single nucleotide polymorphism: A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide – A, T, C or G – in the genome differs between individuals of a biological species or between parental chromosomes in an individual.

Heat shock proteins (HSP): HSP are a class of functionally related proteins involved in the folding and unfolding of other proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress.

Transcriptome: It is the total set of transcripts in a green cell, tissue or organ. A transcript being an RNA molecule reflecting the expression of a specific gene and involved in the synthesis of a specific protein from this gene.

Transcriptomic analysis: it means the analysis of the transcriptome, i.e., analysis of the expression of all genes in a specific cell, tissue or organ.

Dot-blot: A dot-blot is a technique in molecular biology used to detect and quantify a large number of biomolecules, for instance proteins which are present in a green cell, tissue or organ.

Genetic and genomic markers of beef quality

Many genetic markers, including single nucleotide polymorphisms (SNPs) are currently available throughout the world to predict or improve beef quality. However, research in France has shown that the effects of many of these markers are breed-specific. In parallel, research in Ireland validated some markers in Irish crossbred cattle populations but not other markers. However, research in Ireland also identified novel SNP markers that discriminate tenderness from intermediate/tough beef. These markers are available for testing in larger purebred populations. A database of SNP markers with potential to influence beef quality has also been established in Ireland. Further studies are required to identify more appropriate markers for the major pure cattle breeds.

Gene and protein expression profiling of the bovine muscle tissue in France revealed that the expression level of many genes may be potential indicators of muscle mass, tenderness, flavour or marbling of meat. Among them, markers of fat cells are potentially indicators of the ability of animals to deposit intramuscular fat. New markers of beef tenderness have also been identified within the heat shock protein family. In Germany, research revealed major classes of genes which are differentially expressed under n-3 versus n-6 fatty acid feeding regimes.

Using new gene and protein expression tools, a great number of markers of beef quality were identified. However, these markers are often specific to muscle type, animal type, livestock practices or environmental conditions.

Despite these limitations, SNPs or expression levels of genes related to the heat shock protein family, muscle fiber characteristics, metabolic enzymes, connective tissue or proteases involved in ageing of beef may be potential markers of beef quality and in particular of tenderness. Taken together, these results indicate that specific adaptations of predictive tests of beef quality or specific equations of prediction using genetic or genomic tools according to bovine breed, rearing practices, animal type and beef cut would be necessary.

Dot blot

In addition to many genotyping platforms existing in different countries, a bovine muscle gene expression service is now available for stakeholders. Similarly, a dot-blot* quantitative tool for the analysis of several proteins in many bovine muscle samples will be available soon.
Background and need for data/technology/innovation

The beef industry is asking for muscular markers to predict the ability of animals to produce consistent and/or high quality beef.

Beef meat is characterized by high levels of inconsistency in quality, which is an important reason for consumer dissatisfaction. Since most aspects of meat quality are not assessed routinely outside the research setting, it was not possible to incorporate meat quality into animal selection programmes worldwide or simply to predict beef quality using existing knowledge. Therefore, the beef industry is asking for muscular markers to predict the ability of animals to produce consistent and/or high quality beef.

This is compounded by a lack of financial incentives to stakeholders of the beef chain towards improvements in quality. For industry, there is added value to be captured with recent evidence showing of the beef chain towards improvements in quality. For industry, this willingness to pay for improved quality is also borne out in international studies including Norway and recent observations in France. However, at present, retailers lack tools to inform consumers about palatability.

There is thus, a need for tools to discriminate beef on the basis of tenderness and select sires for improved tenderness. Thanks to the development of genomics, recent research has led to the identification of markers for muscle growth potential of bovines, for tenderness and marbling of beef and for traceability of grass-based systems. New tools (DNA or protein chips) are in development to assess a great number of these markers simultaneously. The development of such tools for prediction of quality would be hugely advantageous for the industry and would have a wide range of potential applications spanning animal production via genome-assisted selection programmes, grading and meat management systems.

Experimental studies

A literature review was carried out which permitted us to establish a list of candidate DNA markers with potential to influence beef quality. As part of nationally funded projects, selected candidate markers from the literature were studied in Irish crossbred cattle populations. They were tested for their influence on meat quality traits in M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) muscles. Further novel candidate genes whose expression levels correlated with beef quality were also identified. Some of these genes were sequenced and novel DNA markers (SNP) were identified and tested for association with beef quality.

Similarly, selected candidate polymorphisms were genotyped in French pure breed young bulls and tested for their association with meat quality traits in M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) muscles. Further novel candidate genes whose expression levels correlated with beef quality were also identified. Some of these genes were sequenced and novel DNA markers (SNP) were identified and tested for association with beef quality.

DNA Chip

Different sensory traits to be identified. These technologies were applied to study gene and protein expression in muscle samples from existing nationally-funded experiments (Cassar-Malek et al., 2009) and especially the French MUGENE programme (Hocquette et al., 2007) in which Charolais young bulls and steers were studied, and the LIPIVUMUS programme in which the influence of stress on beef quality was studied (Cassar-Malek et al., unpublished results).

During this research, new molecular tools were developed such as a DNA chip with specific genes involved in muscle biology or beef quality (Hocquette et al., 2010) and a dot-blot quantitative tool for the analysis of several proteins in many samples (Guillemin et al., 2009 and 2011).

Main results

In Ireland (M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) muscles analyzed in crossbred cattle populations).

- A database of in-house research and associations reported in the literature has been compiled.
- One SNP in CAPN2 was confirmed to be associated with tenderness in LTL.
- Reported associations of SNP in DIGAT2, FABP4, TG and leptin genes with intra-muscular fat content were not confirmed.
- SNP in CAST, PRKAG2, CLE and SCD genes were found to be associated with important aspects of muscle technological and sensory quality.
- Novel single nucleotide polymorphisms (SNP) were identified in the AMR2 gene promoter and were found to discriminate tenderness from intermediate/tough beef in France (M. longissimus thoracis et lumborum (LTL) muscle analyzed in pure breed cattle populations).

- A database of in-house research and associations reported in the literature concerning gene and protein expression studies has been compiled (protection under the number IDIN FR.001.2650110.000.K.E.2010.000.10500).
- A specific DNA chip has been developed to routinely assess the expression of genes involved in muscle biology and related to beef quality.
- A dot-blot quantitative tool for the analysis of several proteins in many samples has been developed. Abundance of 24 proteins was quantified by dot-blot and correlated with tenderness in three breeds.
- Protein and gene expression were shown to be highly influenced by rearing practices, environmental conditions, animal type (young bulls vs steers) and/or muscle type.
- A relationship between the transcriptome in two bovine muscles and indicators of physiological and metabolic pre-slaughter stress has been demonstrated. This indicates degradation in nutritional and sensory quality of beef.

Key conclusion or impact for beef stakeholder

Using new gene and protein expression tools, numerous markers of beef tenderness was identified

Nationally funded research in Ireland identified markers in the structural gene that discriminate tender from intermediate/tough beef. These findings are significant since few DNA markers of tenderness are available. Further studies to validate these markers in purebred populations would be valuable. The research carried out in Teagasc through nationally funded projects was compiled with data from the literature in a database of SNP markers with potential to influence beef quality.

Nationally funded research in France showed that the effects of the genetic markers which are currently available to improve beef quality are breed-specific and cannot be extended to all bovine breeds. Further studies are also required to identify other more appropriate markers for French beef breeds.

Gene and protein expression profiling revealed that already known genes as well as previously unsuspected genes may be potential molecular indicators of muscle mass and sensory attributes or marbling of meat. For instance, positive correlations between muscle fat content and a marker of adipocyte differentiation (A-FABP mRNA level) were evidenced among animals, which indicate that A-FABP may be a good marker of the ability of bovines to deposit intramuscular fat. Furthermore, previous French programs described new genes associated with beef tenderness. One of these results concerning a member of the heat shock protein family (DNAJ family) was patented (Genomic marker for meat tenderness, Patent EP063009453, 12/09/2006, WO/2000/023253, Bernard et al., 2007). Expression of DNAJ in bovine muscles according to developmental age and management factors was recently studied (Cassar-Malek et al., 2011). Recent studies in Germany revealed that three major classes of genes were differentially expressed under n-3 versus n-6 fatty acid based feeding regimes; genes encoding lipogenesis related transcription factors and enzymes, intracellular lipid storage associated proteins and cell function and signalling associated proteins (Hiller et al., 2011).

Using new gene and proteinexpression tools, numerous markers of beef tenderness was identified but they are often specific to muscle type, animal type, livestock practices or environmental conditions.
Background and need for data/technology/innovation

**The beef industry is asking for muscular markers to predict the ability of animals to produce consistent and/or high quality beef.**

Beef meat is characterized by high levels of inconsistency in quality, which is an important reason for consumer dissatisfaction. Since most aspects of meat quality are not assessed routinely outside the research setting, it was not possible to incorporate meat quality into animal selection programmes worldwide or simply to predict beef quality using existing knowledge. Therefore, the beef industry is asking for muscular markers to predict the ability of animals to produce consistent and/or high quality beef.

This is compounded by a lack of financial incentives to stakeholders of the beef chain towards improvements in quality. For industry, there is added value to be captured with recent evidence showing that consumers will pay a premium for product of defined high quality. There is thus, a need for tools to discriminate beef on the basis of tenderness and select sires for improved tenderness. Thanks to the development of genomics, recent research has led to the identification of markers for muscle growth potential of bovines, for tenderness and marbling of beef and for traceability of grass-based systems. New tools (DNA or protein chips) are in development to assess a great number of these markers simultaneously. The development of such tools for prediction of quality would be hugely advantageous for the industry and would have a wide range of potential applications spanning animal production via genome-assisted selection programmes, grading and meat management systems.

**Experimental studies**

A literature review was carried out which permitted us to establish a list of candidate DNA markers with potential to influence beef quality. As part of nationally funded projects, selected candidate markers from the literature were studied in Irish crossbred cattle populations. They were tested for their influence on meat quality traits in M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) muscles. Further novel candidate genes whose expression levels correlated with beef quality were also identified. Some of these genes were sequenced and novel DNA markers (SNP) were identified and tested for association with beef quality.

Similarly, selected candidate polymorphisms were genotyped in French pure breed young bulls and tested for their association with meat quality traits in M. longissimus thoracis et thoracis (LTL) within the national funded French QUALVIGENE programme. The biological characteristics of muscle which determine beef quality are controlled by gene and protein expression. Knowledge of these genes and their expression profiles would provide a better understanding of muscle physiological processes and their influence on meat quality. Thus, microarray and proteomics technologies enable multiple genes associated with variation in different sensory traits to be identified. These technologies were applied to study gene and protein expression in muscle samples from existing nationally funded experiments (Cassar-Malek et al., 2009) and especially the French MUGENE programme (Hocquite et al., 2007) in which Charolais young bulls and steers were studied, and the LIPIVIMUSE programme in which the influence of stress on beef quality was studied (Cassar-Malek et al., unpublished results).

During this research, new molecular tools were developed such as a DNA chip with specific genes involved in muscle biology or beef quality (Hocquite et al., 2010) and a dot-blot quantitative tool for the analysis of several proteins in many samples (Guillemim et al., 2009 and 2011).

**Main results**

In Ireland (M. longissimus thoracis et lumborum (LTL) and M. semimembranosus (SM) muscles analyzed in crossbred cattle populations).

- A database of in-house research and associations reported in the literature has been compiled.
- One SNP in CAPN2 was confirmed to be associated with tenderness in LTL.
- Reported associations of SNP in DIOAT, FABP4, TG and leptin genes with intra-muscular fat content were not confirmed.
- SNP in CAST, PRKAG3, GHR and SCD genes were found to be associated with important aspects of muscle technological and sensory quality.
- Novel single nucleotide polymorphisms (SNP) were identified in the ANK2 gene promoter and these were found to discriminate tender from intermediate/tough beef.

In France (M. longissimus thoracis et lumborum (LTL) muscle analyzed in pure breed cattle populations).

- A database of in-house research and associations reported in the literature concerning gene and protein expression studies has been compiled (protection under the number IDDIN.FR.001.260011.000.R.C.2010.000.10300).
- A specific DNA chip has been developed to routinely assess the expression of genes involved in muscle biology and related to beef quality.
- A dot-blot quantitative tool for the analysis of several proteins in many samples has been developed. Abundance of 24 proteins was quantified by dot-blot and correlated with tenderness in three breeds.
- Protein and gene expression were shown to be highly influenced by rearing practices, environmental conditions, animal type (young bulls vs steer) and/or muscle type.
- A relationship between the transcriptome* in two bovine muscles and indicators of physiological and metabolic pre-slaughter stress has been demonstrated. This indicates degradation in nutritional and sensory quality of beef.

**Key conclusion or impact for beef stakeholder**

**Using new gene and protein expression tools, numerous markers of beef tenderness were identified**

Nationally funded research in Ireland identified markers in the structural gene that discriminate tender from intermediate/tough beef. These findings are significant since few DNA markers of tenderness are available. Further studies to validate these markers in purebred populations would be valuable. The research carried out in Teagasc through nationally funded projects was compiled with data from the literature in a database of SNP markers with potential to influence beef quality.

Nationally funded research in France showed that the effects of the genetic markers which are currently available to improve beef quality are breed-specific and cannot be extended to all Iberian-taurine breeds. Further studies are also required to identify other more appropriate markers for French beef breeds.

Gene and protein expression profiling revealed that already known genes as well as previously unsuspected genes may be potential molecular indicators of muscle mass and sensory attributes or marbling of meat. For instance, positive correlations between muscle fat content and a marker of adipocyte differentiation (A-FABP mRNA level) were evidenced among animals, which indicate that A-FABP may be a good marker of the ability of bovines to deposit intramuscular fat. Furthermore, previous French programs described new genes associated with beef tenderness. One of these results concerning a member of the heat shock protein family (DNAIAA; gene) was patented (Genomic marker for meat tenderness; Patent EP06300943.5, 12/09/2006, WO/2005/023335, Bernard et al., 2007). Expression of DNAIAA in bovine muscles according to developmental age and management factors was recently studied (Cassar-Malek et al., 2011). Recent studies in Germany revealed that three major classes of genes were differentially expressed under n-3 versus n-6 fatty acid based feeding regimes: genes encoding lipoxygenase related transcription factors and enzymes, intracellular lipid storage associated proteins and cell function and signalling associated proteins (Hiller et al., 2011).

Using new gene and proteome expression tools, numerous markers of beef tenderness were identified but they are often specific to muscle type, animal type, livestock practices or environmental conditions.

*Continued...
Key Conclusion continued

However, genes or proteins related to the heat shock protein family, muscle fiber characteristics or metabolic enzymes may be potential markers of beef tenderness. Taken together, these results suggest there is now a requirement to adapt or modify genomic prediction tests for beef quality to specific rearing practices, bovine breed, animal type and beef cut.

Following from this research, the private company IMAXIO now offers a bovine muscle transcriptomic analysis service on muscle samples from alive animals for academic or professional partners. The dot-blot quantitative tool for the analysis of several proteins in many muscle samples will be soon available for stakeholders as well.

Main scientific publications:

Aislan, G., Sweeney, T., Muller, A.M., Hamill, R.M. (2010) ‘Regulatory polymorphisms in the bovine Myostatin gene promoter are associated with tenderness and intramuscular fat content’ BMC Genetics, n.11


Dot blot: A dot-blot is a technique in molecular biology used to detect and to quantify a large number of biomolecules, for instance proteins which are present in a given cell, tissue or organ.

Glossary

Single nucleotide polymorphism: A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide - A, T, C or G – in the genome differs between individuals of a biological species or between paired chromosomes in an individual.

Heat shock proteins (HSP) are a class of functionally related proteins involved in the folding and unfolding of other proteins. Their expression is increased when cells are exposed to elevated temperatures or other stress.

Transcriptome: It is the total set of transcripts in a given cell, tissue or organ; a transcript being an RNA molecule reflecting the expression of a specific gene and involved in the synthesis of a specific protein from this gene.

Transcriptomic analysis: It means the analysis of the transcriptome, i.e. analysis of the expression of all genes in a specific cell, tissue or organ.

Many genetic markers, including single nucleotide polymorphisms (SNPs) are currently available throughout the world to predict or improve beef quality. However, research in France has shown that the effects of many of these markers are breed-specific. In parallel, research in Ireland has validated some markers in Irish crossbred cattle populations but not other markers. However, research in Ireland also identified novel SNP markers that discriminate tenderness from intermediate/tough beef. These markers are available for testing in larger purebred populations. A database of SNP markers with potential to influence beef quality has also been established in Ireland. Further studies are required to identify more appropriate markers for the major pure cattle breeds.

Gene and protein expression profiling of the bovine muscle tissue in France revealed that the expression level of many genes may be potential indicators of muscle mass, tenderness, flavour or marbling of meat. Among them, markers of fat cells are potentially indicators of the ability of animals to deposit intramuscular fat. New markers of beef tenderness have also been identified within the heat shock protein family. In Germany, research revealed major classes of genes which are differentially expressed under n-3 versus n-6 fatty acid based feeding regimes.

Using new gene and protein expression tools, a great number of markers of beef quality were identified. However, these markers are often specific to muscle type, animal type, livestock practices or environmental conditions.

Despite these limitations, SNPs or expression levels of genes related to the heat shock protein family, muscle fiber characteristics, metabolic enzymes, connective tissue or proteases involved in ageing of beef may be potential markers of beef quality and in particular of tenderness. Taken together, these results indicate that specific adaptations of predictive tests of beef quality or specific equations of prediction using genetic or genomic tools according to bovine breed, rearing practices, animal type and beef cut would be necessary.