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Functional genomics and new markers for beef production – minireview[1]

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Beef is characterized by a high and uncontrolled variability of its quality which is one reason for the dissatisfaction of consumers. Therefore, the beef industry is asking for muscular markers to predict the ability of animals to produce high quality beef. Thanks to the development of genomics, some recent research allowed the identification of markers for muscle growth potential of bovines, for marbling and tenderness 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.

KEY WORDS : beef / cattle / transcriptomics / proteomics / muscle

In the context of sustainable agriculture, there is a need for new knowledge in order to develop animal farming systems that respond to the new and diversified consumers' demand. It concerns the production of safe high-quality meat products while respecting animal health and welfare, and protecting the environment. Intrinsic quality attributes of beef, and especially tenderness, depend on one hand on *post mortem* factors associated with ageing and cooking, and on the other on muscle characteristics of live animals, which themselves depend on gene expression. Expression levels of these genes as well as interaction between them can now be assessed thanks to the development of functional genomics (e.g. DNA microarrays and proteomic tools)

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– for reviews see Hocquette [2005], Hocquette *et al*. [2009b]. Functional genomics is also expected to be helpful for the beef industry which is looking for biological or molecular indicators that would identify live animals with desirable quality attributes, in order to target them towards the most appropriate production system(s) – for reviews see Hocquette *et al*. [2007] and Cassar-Malek *et al.* [2008]. The strategy is to identify genes or proteins that are expressed differentially between extreme animals without any prior knowledge of the processes involved.

Some of the people working in the beef industry are looking for genes with the aim to sort animals of a high potential for accumulating intramuscular fat in order to produce marbled meat – for review see Hocquette *et al*. [2010]. This has resulted in Japanese and Australian findings concerning Japanese Black and Holstein breeds revealing genes associated with meat marbling [Wang *et al*. 2005]. Other studies allowed the detection of differentially expressed genes (e.g. *NAT1*, *ICER*) associated with intramuscular fat content which have not been previously suspected to play a role in fat accumulation within muscles [Childs *et al*. 2002]. The A-FABP protein content [Jurie *et al*. 2007] as well as leptin and G6PDH expressions [Bonnet *et al.* 2007] have also been recently proposed as relevant indicators of marbling.

In Europe (for instance in France and Belgium), genetic selection in cattle has been directed towards high muscle development at the expense of fat in order to produce leaner carcasses and increase meat production. Biochemical approaches and genomic studies (transcriptomics and proteomics) – Sudre *et al*. [2005], Bouley *et al.* [2005] – have shown that genetic selection in favour of muscle growth leads to a higher proportion of fast-twitch glycolytic fibers at the expense of slow-twitch oxidative fibers. More recent studies have shown that selection for muscle growth potential is associated with modified expression of some genes involved in growth, and also with increased expression of genes involved in glycolysis [Bernard *et al*. 2009]. These modifications are visible from foetal life onwards [Picard *et al*. 2006]. Furthermore, changes in muscle metabolism may be dissociated, at least in part, from fat deposition and beef quality [Bernard *et al.* 2009]. Comparison of the proteomes between the *semitendinosus* muscles of two groups of Belgian Blue bulls with, or without myostatin deletion demonstrated that Troponin T isoform expression was altered by myostatin loss-of-function and could also be a good marker for the prediction of muscle mass [Bouley *et al*. 2005]. In addition, the proteome and transcriptome profiles demonstrated a shift towards a fast-twitch glycolytic muscle type in animals with a myostatin deletion.

Only a few studies aimed at identifying differentially expressed genes according to beef sensory quality (tenderness, juiciness, flavour). Tenderness is the top-priority quality attribute for beef and there is still no simple, reliable and reproducible reference technique to predict it. In the context of the MUGENE programme and in partnership with the French beef industry, the muscle transcriptomes and proteomes were compared on the basis of sensorial quality and shear force for the meat when grilled at 55°C. It was found that expression of the *DNAJA1* gene was negatively related

to tenderness after 14 days of ageing (patent EP06300943.5) – Figure 1. The gene encodes a chaperone protein of the "heat shock" protein family (*i.e*. hsp40) – Bernard *et al*. [2007]. Further, the expression levels of other stress-related proteins (especially HSPB1, which encodes Hsp27) were positively correlated with shear force at either the mRNA or protein level, which confirms earlier results obtained by Morzel *et al*. [2008]. Elevated expression of these proteins, which play anti-apoptotic role in muscles, may potentially slow down the processes of cellular death and consequently meat ageing, thus having a negative impact on the *post mortem* tenderization of meat – for review see Ouali *et al.* [2006]. However, a study carried out on several beef (Charolais and Limousin) and hardy (Salers) breeds showed that potential markers for tenderness appear to differ from one breed to another. Differences in expression of several proteins (parvalbumin, MLC2, ACBP) related to calcium metabolism were found between tenderness groups in the two beef breeds, but not in Salers cattle [Bouley *et al*. 2004].

Proteomic analysis is also used to track protein changes during meat ageing. In beef, the expression levels most affected during the first 24 hours post slaughter are those of HSP and the energy and protein metabolism enzymes [Jia *et al*. 2007]. Moreover, the presence of some HSP27 isoforms in fresh muscle and fragments of this protein in the muscle during ageing explain up to 91% of the variation in sensorial tenderness [Morzel *et al*. 2008].

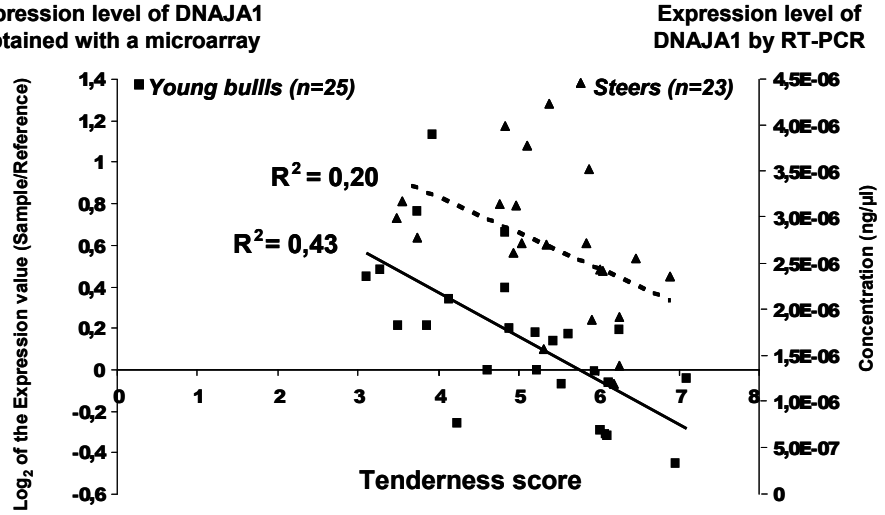

Fig. 1. Relationship between tenderness score of beef and the expression level of the *DNAJA1* gene in the *longissimus thoracis* muscle for Charolais young bulls (from transcriptomic studies) and for Charolais steers (by RT-PCR). This figure is adapted from Bernard *et al.* [2008].

Traceability of an animal's breed and identity, geographical origin, diet and production system are increasingly important factors considered by consumers. A study performed at INRA examined the influence of two production systems (pasture

vs. maize silage indoors) on muscle gene profiles in 30-month-old Charolais steers. The muscles from grazing steers showed more oxidative characteristics than those of steers fed maize silage indoors. An interesting finding was the decreased expression of selenoprotein W in grazing steers [Cassar-Malek *et al*. 2009]. Although its metabolic function is not yet known, selenoprotein W is likely to play a role in the defence processes against oxidants. Thus, muscle selenoprotein W expression could be proposed as a putative indicator for a pasture-based system.

In the context of sustainability of breeding systems, controlling the zootechnical performance of animals is of major economic importance. In this field, genomics techniques provide another viewpoint with respect to the molecular links between nutrition and physiology, and more particularly to the interactions between genes and nutrients. An Australian study demonstrated that after 114 days of under-feeding, many genes corresponding either to structural proteins, or to extracellular matrix proteins or to energy metabolism enzymes, are under-expressed, indicating relative atrophy of rapid glycolytic muscle fibres [Lehnert *et al*. 2006].

In order to validate the relation of the markers previously described to beef tenderness on a large population of meat-producing bovines, it is necessary to have large-scale and trusty techniques. Two methodologies are being developed.

The first aims at quantifying the expression of genes in relation to muscle growth and beef quality. To achieve this goal, we developed an Agilent chip with specific probes of the bovine muscular genes known as predictors of beef quality. More than 3000 genes involved in muscle biology or meat quality were selected from genetic, proteomic or transcriptomic studies, or from scientific publications. As possible, several probes were used for each gene (for example, 18 probes for *DNAJA1*). RNA from *longissimus thoracis* muscle samples of limousin young bulls or of Charolais young bulls or steers slaughtered in 2003 or 2005 was hybridized on the chips. Statistical analyses allowed to select the genes associated with beef tenderness. All the *DNAJA1* probes gave similar results and confirmed earlier findings in Charolais animals slaughtered in 2003, but not in those slaughtered in 2005. With Limousin young bulls we observed a negative correlation of *DNAJA1* expression with calpastatin muscle content (r = -0.30) but not with the other data. The expression of other genes belonging to the same family as *DNAJA1* or linked to other metabolic pathways was associated with beef tenderness. Therefore, numerous markers of beef tenderness can be identified, but they are often specific of an animal type (steer or young bull), of a breed, or of environmental conditions related to the year. However, some gene families (including that of *DNAJA1*) seemed associated with beef quality in different contexts [Hocquette *et al*. 2009a]. The IMAXIO Company will soon propose in service the transcriptomic analysis of bovine muscles[1]

[1]IMAXIO offers high throughput microarray services for many genomic and transcriptomic applications including array comparative genomic hybridization (CGH array), miRNA and gene expression studies. This work was funded by APIS-GENE, INTERBEV, l'Office de l'Elevage now FranceAgriMer and the Regional Council of Auvergne in France.

The second technology aims at assessing different amount of protein related to tenderness. In a first study, we compared Western-Blot and Dot-Blot techniques. The Dot-Blot technique with fluorescence detection presents numerous interests: it allows a good reproducibility and permits the simultaneous analysis of a large number of samples. A very important point is that about 5 to 10 animals per group are required to detect large differences (>1.5) in biomarker expression between tender and tough beef, whereas much higher numbers of animals (10 to 30) are required to detect smaller differences (1.2 to 1.3) taking into account the biological variation of these markers [Guillemin *et al*. 2009]

In conclusion, thanks to the development of powerful technologies, genomics is reshaping physiology. Gene expression profiling has revealed that unsuspected genes may be potential molecular indicators of muscle mass and sensory attributes or marbling of meat. The question is now how to modulate them in order to increase beef quality. In addition, various biotechnology tools are being developed to assess routinely and simultaneously all the genes known so far to be involved in beef quality. Simultaneously, we are looking for SNPs in those genes to assess their potential association with beef quality within the QUALVIGENE programme, which is another programme funded by the French Beef Industry.

Genomic techniques have also resulted in increasing number of data which now need to be stored and interpreted by association with phenotypic observations. The first problem to be dealt with is creation and management of appropriate databases. The second is the need for an ontology system for phenotypic data [Hughes *et al.* 2008]. The third problem is how to characterize phenotypes in less costly, standardized and rapid fashion in order to make the most of the increasing flow of genomics data. High-throughput phenotyping is currently being developed in the mice by the International Mouse Knockout Consortium in order to better characterize different mutated animals [Brown *et al*. 2005]. At the forefront are Australian researchers, since they have developed the most comprehensive predictive meat quality grading system ("Meat Standards Australia") available to date. In order to sharpen the system's performance, they intend to integrate new genetic and genomic markers currently in the validation phase. This integrative view is the key to meeting today's challenges. It is also an objective of the EU-funded programme entitled ProSafeBeef (http://www.prosafebeef.eu/asp/).

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Genomika funkcjonalna a nowe markery genetyczne w produkcji wołowiny

S t r e s z c z e n i e

Jakość wołowiny może być bardzo różna i jest trudna do kontrolowania, co często bywa przyczyną niezadowolenia konsumentów. Dlatego przemysł mięsny chętnie widziałby opracowanie genetycznych markerów pozwalających na wczesne wytypowanie zwierząt – producentów wołowiny o wysokiej jakości. Dzięki najnowszym osiągnięciom genomiki możliwa jest obecnie identyfikacja markerów dla takich cech jak rozrost umięśnienia, zawartość tłuszczu śródmięśniowego (marmurkowatość), kruchość mięsa, a nawet markerów do identyfikacji mięsa pochodzącego od zwierząt chowanych w systemie naturalnym (pastwiskowym). Opracowywane są nowe narzędzia, oparte na technikach microarray, umożliwiające równoczesne oznaczanie wielu takich genetycznych markerów u wybranych zwierząt dla oceny ich wartości hodowlanej pod względem jakości wołowiny.