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Applications of proteomics and genomics for improving meat quality

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Abstract

This paper introduces a series of phenotypic analyses done in parallel with genotypic analyses for the bovine meat quality. This has allowed new knowledge to be gained of the genetic, environment and management factors that impact on the carcase and eating quality, visual appeal, odour and health attributes of meat quality. The research described involved close collaboration with commercial partners across the supply chain in the sire breeding as well as the meat processing industries. This approach has enabled timely delivery and adoption of research results to industry in an unprecedented. A special attention will be given to meat production, as well as quality control. In the latter, a way and provides a good model for future research. Sufficient diversity in performance and adaptability can be exploited for actual improvement accruing to conservation and development of indigenous cattle resources.

Key words : Genomics, meat quality, markers, Proteomics.

INTRODUCTION

Meat quality traits are related to the biological traits of the live animal, hence biological sciences including genetics, physiology, cell biology and biochemistry has been widely employed for decades to identify the biological mechanisms behind meat quality traits, like fat content, tenderness, and water holding capacity of meat, but also with special attention on muscle growth, development, and carcass composition. Adipose tissue has important role on meat quality[1,2].It is clear that meat quality traits are complex and multigenic in nature, hence detailed characterization would benefit from, experimental approaches and technologies aimed at analyses of various genes and proteins is influenced by several factors, such as breed, genotype, feeding, fasting, preslaughter handling, stunning, slaughter methods, chilling and storage conditions[3].For the genetic basis, the correct selection of breeds is necessary because the genetic influence on meat quality is very different among breeds as well as among animals in the same breed. Such strong selection, especially in recent centuries, has resulted in the accumulation of new mutations with favorable phenotypic effects [4].These new mutations can provide greater options, especially in molecular technology. Many factors affect the quality of meat, including the way animals are fed, managed, slaughtered and both carcass handling and processing postslaughter. With the help of Proteomics we are enabled to describe the modification of postmortem protein of pig muscle protein [5] and also sarcoplasmic protein and variation in meat color can be investigated [6].While there is often emphasis on the management systems that can be implemented to meet market specifications there has, until recent years, been little emphasis on factoring in the molecular or biological components of meat quality. Few studies has reported on proteome of bovine adipose tissue [7,8].Although, after compensatory growth by restricted feeding in pigs through proteome analysis of muscles[9].We are now in an exciting period where many new opportunities are provided to researchers through the application of genomics, proteomics and other approaches. Important traits for meat quality that may benefit from MAS include meat pH, marbling and tenderness [3].There are several approaches to identifying markers for MAS. The candidate gene approach begins with an examination of the physiological pathways underlying the trait. Sequencing phenotypically divergent individuals at candidate loci may lead to the identification of single nucleotide polymorphisms (SNP's) or insertions/deletions that can be investigated for associations with traits of interest. In the second approach, a mapping population of pedigrees (usually an inter-breed cross) is selected in which the phenotype of interest is segregating and the genes (QTL's) for all the indigenous cattle of Pakistan belong to zebu (humped type) cattle (Bos *indicus*). There are 15 recognized breeds of cattle in the country which constitute 43% of the total cattle population. In the absence of any pure breeding programme (with the exception of Sahiwal conservation project), for example, populatin of purebreds was expected to decrease since the 1996 livestock census but it has increased by about 10%. It is interesting that purebreeds of all the species have been indicated to increase at about the same rate in the 2006 livestock census. Achai, Gabrali and Cholistani are the new entries in the 2006 livestock census and are now expected to stay as breeds. An important cattle breed, Dajal is still missing in the census list because it is not available as purebred at any Government livestock farms and is likely to be vulnerable to depletion. Out of various breeds available in the country, Red Sindhi and Sahiwal are well known internationally as tropical dairy cattle breeds. Both have been used for producing new breeds. A recent FAO report indicated that Sahiwal had been taken to12 African countries (FAO,2007). Although population of Sahiwal in India is not very significant(restricted mainly to Government farms), aggressive marketing campaign by countries like Australia to sell Sahiwal still goes on. Cholistani and Tharparkar are other two important breeds with dairy production potential than many other breeds. Draft cattle breeds include Bhagnari, Dajal, Dhanni, Kankraj and Rojhan. Population of Kankraj in the 2006 livestock census (273 thousands) is quite

unusual (five times that of 1996 census). In practice, the candidate gene approach is often combined with the mapping technique[4].(*MYOD1*) and *myogenic factor 5* (*MYF5*) have been proposed as functional and positional candidate genes for carcass composition and meat quality in livestock [10],[11].

Genomics Tools for recognizing Biomarkers of Tenderness:

Comparative Proteomics

From many years, various proteomic analyses were performed in specific programs to better understand the mechanisms involved in tenderness. However, the strategy has been to compare extreme groups of beef tenderness by proteomics[12], [13] and/or transcriptomics [14].For comparative proteomics, the proteins of muscles from two groups (very tender and not tender) were extracted and separated according to their isoelectric point by two-dimensional electrophoresis.

Effects of Breed on meat quality

It is well-known that genotypes differ in muscle characteristics due to marked differences in their physiology. Simultaneously, beef may differ in quality depending on the animal genotype. That is, meat from Bos *indicus* cattle is less tender than that from Bos *taurus* breeds. The lower tenderness is due to reduced proteolysis of myofibrillar proteins in muscles from *B. indicus,* associated with greater activity of calcium-dependent protease inhibitor [15]. It was also demonstrated that beef breeds (Blonde d'Aquitaine and Limousin) were characterized by lower collagen content, and compression and shear force in raw and cooked meat, compared to dairy (Holstein). The meat quality attributes were measured by panels scoring tenderness, juiciness and flavor of cooked meat. Steaks were grilled to an internal temperature of 70°C. Genetic changes were estimated in 10 publications. The mean heritability coefficient *(h^2)* for the tenderness score was 0.24, while for juiciness and flavor scores as low as 0.11 and 0.09.Somehow, the genetic correlation coefficients *(rG)* between the three scores appeared very high (0.84 to 0.91 on average) suggesting the panel could not really be used to differentiate between the quality attributes. Studies include, that is a mechanical measure of the texture of cooked (70°C) meat, and either grilled (US) or cooked in water bath (Australia).Although, the mean h^2 appeared high (0.26, n = 14) as well as the mean *rG*with tenderness. The developing field of farm animal genomics

Genome research in farm animals develop frequently in recent years, moving from linkage maps to genome sequence. The work on farm animal genome sequencing began in the early 1990s, and assists in the understanding of how genomics function in various organisms [16]. It will be useful in different fields, for instance study the molecular components and improvement of meat quality In March 2004, the first draft of the chicken genome was released [17]. In May 2006, the Genome Sequencing Center submitted an improved 6.6X draft chicken genome assembly. The chicken genome has a haploid size of 1200 Mb. It is not only a food animal that comprises 41% of the meat produced in the world, but also a model organism for studies of disease and biology [18]. With the chicken genome sequence, especially the genome-wide screening in three chicken breeds yielding a set of 2.8 million SNP markers [19], chicken breeders will have a framework for investigating polymorphisms of informative quantitative traits to continue the directed evolution of these species[11]. In October 2004, the first draft of the bovine genome sequence was deposited in a free public database. In June 2005, the Bovine Genome Sequencing Project released the second version of the bovine genome.

Genomics Markers

The genome scans identify studies the relationship between a trait and markers selected across the genome to identify chromosomal locations associated with the trait [2]. The genome scan will find out the map location of a trait locus with a major effect. It include the following steps: (1) design and construction of a resource population, (2) phenotyping traits of the resource population, (3) selection of genetic markers, (4) genotyping of the population for selected markers, (5) construction of linkage maps, (6) statistical analysis of the phenotypic and genotypic data derived from the resource population [20]. The design of a resource population is the first step in genome scanning that will decide whether QTL can be found. A resource population is a population generated for a particular research purpose, with phenotypic information and sufficient DNA supply for genotyping; for instance, an intercross between two divergent breeds of farm animal or a population containing particularly interesting phenotypic data. Because the design of the intercross between two divergent populations of farm animals has a more powerful approach for QTL mapping, it is used in most resource populations, e.g. the wild boar and large white pigs [21]. Three types of observable polymorphic genetic loci can be distinguished: (1) direct markers loci that are the functional mutations, which causative for the trait of interest; (2) LD markers loci that are in linkage disequilibrium across the population with the functional mutation; (3) LE markers loci that are in linkage equilibrium with the functional mutation in outbred populations [22]. The three types of marker loci differ not only in methods of detection, but also in their application in selection programs. Selection on these three types of markers is referred to as gene-assisted selection (GAS), LD markers assisted selection (LDMAS), and LE marker-assisted selection (LEMAS). GAS is currently the most practical and commercially viable system, because GAS gives certainty to the inheritance of the desired trait and so can be used for selection across the population. To LDMAS, the extent of linkage in the genome and the population history decide its utility [17]. Because linkage disequilibrium extends far in cattle breeds [23], it is possible to use markers that are in linkage equilibrium with the QTL in the general population.[17] However, LD markers are difficult to identify and there are only few detected in livestock populations to date [24]. Although, LE markers are readily identifiable, LEMAS is too difficult to use in commercial breeding. LE studies are currently most useful in the initial stages of marker identification, such as finding QTLs that segregate between breeds [25].

The economic advantage of small improvements in production or meat quality traits is important and may be achievable through unravelling the relationship between the genome and these traits. Quantitative trait loci (QTLs) are stretches of DNA that are closely linked to genes that underlie a trait (phenotype). If desirable QTL alleles1 can be identified which have significant physiological associations with meat quality, these may be combined with estimated breeding values (EBV's) and incorporated into best linear unbiased prediction (BLUP) models in a process known as marker assisted selection (MAS)[3]. MAS has particular advantages for traits that challenge traditional selection such as lifetime fecundity or those that must be measured post mortem, such as many meat quality traits [17]. The additional genetic gains to breeding programmes from MAS are greatest for these traits [17]. At present, the increased profit due to

the incorporation of molecular markers in selection programmes is derived mainly from bulls with favourable allelic combinations achieving increased market share of breeding stock[17]. There are a number of organisations currently marketing commercial tests for polymorphisms in genes that are related to particular meat quality phenotypes in beef production. Commercial companies marketing tests include Igenity® Merial, Genetic Solutions and MMI genomics. Tests incorporate markers discovered under research programmes at CSIRO Australia, the U.S.

Role of Proteomics

Proteomics can be defined as the systematic determination of protein sequence, quantity, modification state, interaction partners, activity, subcellular localisation, and structure in a given cell type at a particular time (Nature Biotechnology editorial 2003 Vol 21, p213). Proteome analysis is a direct measurement of proteins in terms of their presence and relative abundance [26]. Neither genomic DNA code nor the amount of mRNA that is expressed for each protein yields an accurate picture of the state of a cell. This is because genes may be present but not transcribed and the number of mRNA copies does not always reflect the number of functional proteins present [27]. The goal of proteomics is to achieve information about cellular protein expression. Proteomics can address problems that cannot be solved by using DNA analysis. As regarded to functional aspects, these problems include estimation of the relative abundance of the protein product, its posttranslational modification, subcellular localization; turnover and interaction with other proteins[28], [29]. There are two approaches to proteome characterisation, namely comparative proteomics and mapping proteomics. Comparative proteomics characterise the biological mechanisms which form the link between observable phenotypes and genotypes, thereby taking moment by-moment snapshots of cellular responses at the protein level [30]. Mapping proteomics is identical to genome sequencing projects and aims to characterize and make comprehensive databases of "cellular proteomes" [31].

Proteomics Tools

Proteomics are the tools which areused to analyses the proteomes. Mostly proteomics tools are based on protein separation in at least two dimensions using either chromatographic methods or electrophoresis, and it is commonly followed by the use of mass spectrometry (MS)[32]-[40]. The presence of high-abundance proteins in a tissue or cell somehow masks low-abundance proteins and thus prevents their detection in proteome studies. The use of pre-fractionation methods can assist in the detection of low-abundance proteins that may finally prove to be informative biomarkers. Various established protein and peptide fractionation techniques include stepwise extractions of proteins, immunodepletion, reverse phase or ion-exchange chromatography and gel Wltration[41].The choice of technique is greatly dependent on which subset of proteins that is of interest. In muscle cells proteins, such as actin and tubulin are high abundant proteins.

Postmortem changes

In most developing countries and traditional meat shops in developed world meats are usually displayed unpackaged. Packaging primal or retail cuts have been achieved by controlling the gas atmosphere (Oxygen, Carbon dioxide and Nitrogen gases) surrounding the meat to produce favorable effects most especially on meat shelf life and appearance. It is reported that packaging has three basic functions that is protecting meat from contamination and inhibiting microbial growth, reducing or eliminating evaporative weight loss[42]. When muscles are cooled below 10°C , cold shortening occur which makes the meat hard upon cooking, and slow freezing may produce cold shortening before freezing whilst rapid freezing may results in thaw rigor [42]. Thaw rigor meat losses large amount of drip or water during thawing and are hard upon cooking. A condition known as heat ring characterized by darker band muscle forming can occur in beef carcasses subjected to relatively fast chilling [42].

Proteomics is helpful to study changes occurring in muscle during *post-mortem* storage. Total protein extracts from pork LD samples collected at 0, 4, 8, 24 and 48 h after slaughter revealed that 15 proteins were changed, some increasing and some decreasing in abundance after slaughter [43], [44]. Several of these proteins were identified as fragments of structural proteins such as actin, myosin heavy chain and troponin. The contribution of proteolysis to meat tenderness is predominantly controlled by the protease levels in the muscle at slaughter, duration of post-rigor ageing and protease activity during ageing [45]. The effect of the calpain system on tenderisation post-slaughter relies on a balance between the rate of activation and activity.

Proteomics future

Proteomics is developing now a day Modern mass spectrometry instruments have a resolution well 10 proteins in plasma make up nearly 90% of the total protein [46]. These 2 factors have led to the addition of various protein separation methods to proteomic experiments atomic mass unit. This allows ready matching of a particular tryptic fragment to a specific amino acid composition, since only a single combination of amino acids will provide a suitable match in probably all cases. Although, this does not generally provide an unambiguous determination of the particular sequence of amino acids, since any sequence combination of the same amino acids match the molecular weight estimates. That's why, automated search routines that compare the tryptic map with that of other proteins are crucial. These maps can be theoretical maps, based on DNA. Technical advances have improved the sensitivity and accuracy of mass spectrometers necessary for proteomic work. Except this high sensitivity, 2 factors complicate protein identification: first, the number of proteins that constitute a proteome, and second, the expression level range. First, the number of proteins that constitute the human proteome is estimated to be greater than 30,000 proteins, not counting alternative splice variants and posttranslational modifications [39]. Second, the range of protein expression complicates detection of low abundance proteins in typical biological samples. The expression dynamic range is estimated to be greater than 7 orders of magnitude [40]. Forexample; nearly half of the protein in plasma is albumin, and the top before mass spectrometry. That's why, different fractionation schemes of the proteome into less complex mixtures are important for a more complete identification of proteins. Fractionation can be achieved by subcellular fractionation, enrichment strategies, chromatography, or gel electrophoresis [40]. These fractionation strategies can be used individually or in combination to improve detection of small abundance proteins. The stresses of parturition and shipping have been clearly shown to suppress the innate immune system in cattle and calves [47]-[49].The research demonstrating stress induced immune-suppression has been accumulated in a large number of detailed experiments. A single proteomics experiment allows an investigator to examine stress models globally, in a search for new or unrecognized innate

immunology pathways that are affected by stress in cattle phenotypic characters of meat quality are affected by many factors[50]-[52].

Postmortem storage times/temperature contribute crucially to meat characteristics in some as yet unknown ways but can be known in future. Proteomic approaches are being used extensively to examine postmortem changes in slaughtered beef.

CONCLUSION

Several novel mutations were identified in the bovine MYOD gene family in this study. Substantial differences in allele frequencies were observed among different cattle breed. These identified SNPs can be used as markers for selection of animals and potentially used for cattle breeding using modern methods, such as marker assisted selection or marker assisted introduction. Somehow, they improve our understanding of the biological mechanisms that determine meat quality and provide elements (markers) to move from knowledge to the development of tools for evaluation of these complex traits. Many exciting discoveries have been made, through investigation of the genome and proteome in relation to meat quality Potential applications of this research encompass improvements to traditional breeding programmes, diagnostic tests for quality and management systems for quality. The development and rapid advances in molecular and quantitative genetics, reproduction technologies, animal nutrition and muscle science carry with them a huge potential. To upgrade advances in molecular genetics have led to the recognition of genes or markers associated with genes that affect the meat quality trait. The molecular basis of meat quality is being revealed by functional genomics approaches. These will help us to achieve further insight into the biological components and the development of meat quality. It gives greater opportunities to enhance genetic improvement program in various fields.

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