AN EUROPEAN INITIATIVE TOWARDS IDENTIFICATION OF GENES CONTROLLING PORK QUALITY

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OBJECTIVES

Recently an EU funded project, PorDictor (QLK5-2000-01363), has been started aiming at the identification of new predictors for pork quality derived from gene expression profiles of skeletal muscle during prenatal development. A consortium of eight academic and industrial partners from four countries, Germany, Italy, The Netherlands and UK, set up a multidisciplinary approach, including molecular biology, developmental biology, breeding science, quantitative genetics, veterinary science and histology, to elucidate the factors that are important for the genetic regulation of prenatal processes and their relation to postnatal muscle characteristics that are important for meat quality. The overall goals of the project are (1) to identify potential functional candidate genes controlling muscle phenotype in terms of technological measurable meat quality traits, (2) to confirm their effect on meat quality and (3) to provide DNA tests suitable for implementation in pig breeding in order to breed for improved pork quality.

BACKGROUND

Meat quality is of economic importance in animal production. Current market surveys show that consumers are increasingly willing to pay extra money for high quality meat. During the past decades pig breeding has focussed on improving lean meat production. An inverse genetic relationship between leanness and meat quality traits has been reported in pigs. Furthermore, meat quality traits can only be measured at *post mortem*. Hence, meeting the two breeding objectives, increase of leanness and improvement of meat quality, is a difficult task. Knowledge of the genetic and developmental biological factors affecting meat quality will

provide a important contribution to overcome the antagonisms and to breed for improved leanness and meat quality traits more effectively.

Meat quality can be characterised from the consumers' perception, and with technological parameters. Important organoleptic parameters that characterise the consumers' perception of pork include taste, toughness, juiciness (eating quality), colour and visible fat content (selling quality). Technologically, meat quality can be described with objective technologically measurable parameters like conductivity, shear force, colour, intramuscular fat content, water binding capacity and pH, which are all of moderate heritability (h^2= 0.15 to 0.30 ; Sellier, 1994). These traits are addressed in the project together with muscle fibre type distribution traits (h^2=0.2 to 0.5 ; Dietl *et al*., 1993), lean meat content and loin eye area (h^2=0.4 to 0.6 ; Sellier, 1994). For breeding purpose the sensory meat properties of consumers´ concern can be translated to objective technological traits. Significant and medium to high correlations were found between tenderness, juiciness, taste (organoleptic parameters) and shear force, pH1, conductivity (technological parameters) (Hovenier *et al*., 1993). Significant correlations (about 0.3) were also found between histo- and biochemical characteristics of M. longissimus dorsi and pork eating quality traits (Henckel *et al*., 1997).

Meat quality traits are to a large extent determined by genetic factors active during prenatal muscle development. Meat quality depends on the composition of muscle tissue where muscle fibres are the major components. Meat quality parameters, like shear force, colour, pH, conductivity, and quantity of meat are directly related to the number and proportion of different types of muscle fibres (Ashmore *et al*., 1973 ; Swatland *et al*., 1973). Significant correlation between muscle fibre structure, their biological properties and meat quality traits have been shown (Wegner and Ender, 1990 ; von Lengerken *et al*., 1994).

Genotype may determine the timing and level of expression of certain genes during prenatal muscle development. The resulting phenotype in number of muscle fibres is fixed at birth, and will influence the final phenotype of muscle at slaughter related to meat quality.

Consequently, the project aims at expression profiling of prenatal muscle tissue in pigs of two extreme breeds in meat quality and muscularity in order to establish a list of candidate genes that will be screened for polymorphism and associated with meat quality traits obtained in commercial pigs at slaughter.

PROGRESS AND ACHIEVEMENTS

Based on histochemical studies on porcine prenatal muscles (Swatland *et al*., 1973 ; Ashmore *et al*., 1973 ; Novakofski and McCusher, 1993), seven key developmental events have been identified that are examined for differential gene regulation *in* and *ex situ*: start of myogenesis (d14), start of terminal differentiation (d21), appearance of primary myotubes (d35), appearance of presumptive slow twitch (beta) fibers (d49), appearance of secondary myotubes (d63), discrimination of two fiber types by myosin ATPase activity (d77), muscle hypertrophy (day 91). At each sampling point about 2000 embryos of the breeds Duroc and Pietrain were collected for RNA extraction and *in situ* studies of whole embryos/fetuses or (presumptive) *M. longissimus dorsi* tissue.

A huge number of genes are expressed in muscle tissue at different times of development all of which more or less contribute to the muscle phenotype. An ever-increasing number of such genes is known mainly from studies in laboratory animals. The project aims at the characterisation of these genes that are already known to be involved in myogenesis from pigs or other animals and novel genes.

During the first phase of the project several hundred clones representing genes known to be involved in myogenesis were derived from skeletal muscle cDNA libraries for further expression profiling. In parallel, meat quality traits including pH, conductivity, colour, shear force and drip loss were recorded from fulsibs of the foetuses allowing to estimate breeding values. Phenotypic studies of the foetuses by *in situ* hybridisation, and immunohistochemistry already revealed new insights into the morphological and biochemical changes in muscle at different stages of gestation and indicate that embryonic, perinatal and slow myosin heavy chains are co-expressed in early gestation while segregation into distinct fibre types occurs later in gestation.

In the next phase of the project the known candidate genes will be examined for their breed-specific, stage-specific and phenotype-associated expression by DNA chip technology together with novel functional candidates. Novel candidates will be derived by expression profiling during prenatal muscle development of animals of two breeds, Duroc and Pietrain, that are extreme breeds with regard to meat quality and muscularity with the Duroc breed being superior in taste, toughness, juiciness and colour. Several techniques for expression profiling, i.e. cDNA-chips, RT-PCR-ELISA, Northern analyses, Differential Display-RT-PCR, construction of stage-specific muscle cDNA libraries and subtractive hybridisation, will be applied.

The genes from the shortlist of breed-specific, stage-specific and phenotype-associated regulated candidates will be screened for polymorphism preferentially in their 3´-UTR by SSCP or comparative sequencing. Simple PCR-tests will be established to genotype at the loci. Finally, performance tested porkers of the commercial crosses Pietrain x Landrace and Duroc x Large White will be sampled together with the phenotypic data (meat quality) and pedigrees of the animals. These animal will be genotyped at the functional candidates and association between the candidate genes and technological meat quality traits will be evaluated in order to end up with a set of DNA tests suitable for implementation in pig breeding programs for improvement of pork quality.

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