

QUANTITATIVE AND MOLECULAR APPROACHES TO MANIPULATE CARCASS COMPOSITION IN FARM ANIMALS: A MAJOR QUALITY DETERMINANT.

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Keywords: Meat quality, quantitative genetics, major genes, gene mapping

THE CONSUMER DEMAND FOR LEAN MEAT

Consumer health concerns with respect to animal product options have been studied extensively in the USA (NRC, 1988; Huffman *et al.*, 1991; Keeton, 1991) and elsewhere (Woodward and Wheelock, 1990; Harrington, 1994). In developed countries where meat is a staple part of the diet, increased emphasis is being placed on purchasing meat with a lower fat content (Hopkins, 1988; Thatcher, 1988) while nutritional research is emphasizing the beneficial nutritional properties of lean meat (NRC, 1988). As a result, pressure is increasing to encourage the production of leaner cattle, sheep and pigs and the processing of their carcasses and cuts by trimming excess fat to produce leaner cuts or even lean (completely fat trimmed) beef and pork for retail sale. This demonstrates the increasing pressure from consumers for less fat meat, which has been facilitated by media publicity highlighting the adverse health effects of animal fats and that of meat in general. Despite these clear market signals, the producers of red meat at least have failed to make significant changes to their product. For example, lamb in the USA increased carcass fatness from 1968 to 1988, Harris *et al.* (1990).

In Denmark and elsewhere, retail demand for leaner pork has resulted in national programs for leaner pork production (Fredeen, 1958) in advance of similar fat reduction pressures for other species. Goutefongea and Dumont (1990) reported that the fat content of pork in Finland had dropped 30% over the past 20 years and in the USA the equivalent figure for pork was 15%. Development of a farmed deer industry (Fennessy and Drew, 1985) resulting in increasing sales of venison and also of goat meat (both low fat products) also indicates consumer demand for leaner meats as well as a search for new products. Undoubtedly, continued media publicity on the negative aspects of a high fat diet and advice from nutritionists and expert panels have resulted in a decline in the consumption of red meat in particular.

GENETIC SELECTION

In cattle, multivariate predictions based on the extensive within-breed genetic parameters for the Charolais by Renand *et al.*, (1994) showed that selection for improved growth can be expected to increase leanness and to reduce fat. Furthermore, selection for high lean and low fat simultaneously (i.e., for lean growth rate, or strictly, lean % minus fat %), would reduce by

about 50% the gains that could otherwise be achieved in carcass weight-for-age, which is closely in line with evidence from sheep. Renand (1995) concluded that the contrast between the lean and fat contents of beef carcasses was the major determinant of genetic variability in carcass quality. The data of Renand *et al.*, (1994) indicate that some degree of genetic independence between fat and lean exists in cattle (genetic correlation = 0.66) as well as in pigs (Wood *et al.*, 1994). Other similar evidence from the scientific literature has been reviewed by Kemp (1994), who reported an average heritability of 0.4 for marbling score in beef cattle.

Characteristic breed differences in fat partitioning to subcutaneous versus intra-muscular depots have been reported for sheep and cattle (Kemp 1994; Renand *et al.* 1994), as well as for pigs. Fast-growing, lean European "white" pigs show low carcass marbling compared with dark-skinned Durocs and the fatter American breeds according to Wood *et al.* (1994). It is noteworthy that the Duroc's high concentration of marbling relative to backfat is associated with increased tenderness, juiciness and redness, and other changes in muscle characteristics, which are suggestive of an increase in the concentration of red oxidative muscle fibres (Wood *et al.*, 1994). Such results point to characteristic associations of eating qualities with lean, as well as with fat, and at the level of individual muscles as well as at the carcass level.

Reduction of fat by genetic means is expected to have an influence beyond that of total carcass fat content and to impact on quality features of the lean component of carcass weight as well. Recent multivariate predictions based on within-breed genetic parameters for Charolais cattle (Renand *et al.*, 1994) show that selection for improved growth is expected to reduce the intra-muscular fat content of the longissimus muscle, increase its ultimate pH and collagen content, reduce the solubility of the collagen and increase the oxidative activity and diameter of its muscle fibres. Selection for lean growth rate would be associated with similar changes in ultimate pH but reduced collagen and haem iron contents in the longissimus muscle, increased collagen solubility and fibre size, as well as greatly enhanced responses in carcass lean content. It would also be genetically associated with an increased glycolytic activity of this muscle. The restrictions on carcass growth needed to control concomitant changes in intra-muscular fat were minimal and were genetically associated with a metabolic profile towards low lactate dehydrogenase enzyme activities and high isocitrate dehydrogenase activities (i.e., a strong metabolic shift from glycolytic to oxidative fibre types). The proportion of heavy chain myosins would also increase, especially if changes in collagen content were to be avoided as well (Clarke *et al.*, 1996).

Such physiological changes from selection for reduced fat content could also impact on production and processing aspects of meat production. Some of these seem likely to be associated with concomitant changes in protein accretion, which seems more likely to arise from decreased rates of proteolysis than from increased rates of protein synthesis (Klasing *et al.*, 1987; Baldwin *et al.*, 1991; Goldspink, 1991). For sheep selected over several generations and widely divergent in weaning weight, Oddy *et al.* (1995) demonstrated that genotypic differences in the rates of protein gain were almost exclusively driven by the rate of protein degradation. Evidence from the plasma metabolic profiles of sheep selected for and

against lean growth rate also point to genetic variations in protein degradation (Cameron and Cienfuegos-Rivas, 1994). Scheurs (1995) reported a markedly decreased proteolytic capacity of both the calpain and cathepsin enzyme systems in chickens selected for fast growth, while chickens with efficient protein metabolism showed intermediate calpain/calpastatin values but increased cathepsin and cystatin activities. Both strains of chickens showed similar rates of pH decline post-mortem in commercial broilers, and all were higher than the protein-inefficient Leghorn chickens used for egg production. Leghorns showed higher myofibrillar fragmentation indices and lower shear force values in breast muscle than the chickens selected for growth rate. The protein efficient line showed lower shear force values than Leghorns and broilers, which may need ageing times longer than 24 hours.

MAJOR GENES

Although important gene variants have been identified, most of the identified single gene effects on carcass composition for livestock are more directly associated with traits other than fat content. Gene effects on proteolysis and double muscling have, however shown important associated effects on fat.

The Callipyge Gene in Sheep. The callipyge gene in sheep first became apparent in an American Dorset flock selected with emphasis on leg conformation. Shay *et al.* (1995) have confirmed the location of the gene on chromosome 18 of the sheep in the interval spanned by the markers CSSM18 and TGLA122. Some unusual inheritance features suggesting dominant negative imprinting are evident (Cockett *et al.*, 1995). The gene has pronounced effects on muscle development. Animals display increased muscle size in the loin and leg, leaner carcasses, increased feed efficiency, but tougher meat (Carpenter and Solomon, 1995). Recent evidence confirms earlier reports on the callipyge phenotype (Al-Hassan and Roberts, 1995; Brown *et al.*, 1995; Goodson *et al.*, 1995; Hill *et al.*, 1995; Hossner *et al.*, 1995): higher eye muscle area (30-69%), similar slaughter and carcass weights, but higher dressing percentages; lower carcass fat thickness (24-45%), internal fat weights and muscle marbling scores. The gene leads to reduced lung and liver weights and small intestine mass; more efficient liveweight gain under rapid growth, higher nitrogen balance and utilisation of crude protein. There is also higher serum growth hormone and IGF-1, reduced serum insulin (39%) and IGF-1 under restricted feeding (20%), increased circulating IGF BP-2. Higher yields of trimmed cuts and high value cuts from the carcass (by 3-8 percentage points) have been found, and higher but variable muscle weight advantages from the leg and loin regions (20-42%), but not for slow-twitch muscles in the shoulder.

Double Muscling in Cattle. The double-muscling gene has been identified in the Belgian Blue breed of cattle (Hanset and Michaux, 1985), and there is the chance it is also segregating in other breeds, such as Charolais, Limousin and Simmental. The gene leads to higher proportions of high priced cuts in the hind-quarters, which require less fat trim. Hanset *et al.* (1987) found that double muscled Belgian Blue bulls at 12 months of age (relative to conventional Belgian Blue bulls) had a 12 percentage point increase in % lean (70.5 vs

58.7%) and 9 percentage point reduction in the fat content (12.8 vs 22.1%) in the 7th rib-cut. A marker linked to the gene has been identified by Georges *et al.* (1990).

The Acid Meat Gene in Pigs. A major gene (unfavourable dominant), the "acid meat" (RN) gene in pigs, increases the glycogen level and branching enzyme activity in the myofibres, giving rise to a lowered protein content, ultrastructural abnormalities and resulting in decreased technological abilities associated with high lactic acid levels postmortem (Le Roy *et al.* 1994).

In pigs, intense selection since the 1960s for growth efficiency and high meat (and low fat) percentages have had the side-effect of producing "pale, soft and exudative" (or watery) meat. This syndrome was associated with malignant hyperthermia (Webb and Simpson, 1986), the gene is now mapped, and a marker on the ryanodine receptor gene has been identified (Fujii *et al.*, 1991).

GENE MAPPING AND MARKERS

Excess fat in farm animals could be considered analogous to the human condition of obesity. The complexity and diversity of the physiological and environmental pathways leading to excess fat are enormous, but are beginning to be better understood. Comparative gene mapping to date indicates that mammalian genomes in general are highly conserved and that the developing new maps of mammalian species can draw from one another and especially the prototype maps of the mouse and humans (Womack, 1991). Genetic maps are rapidly being constructed as a basis for the identification of markers associated with Quantitative Trait Loci (QTLs) in farm animals used for meat production.

Reverse genetics has been defined as the use of linked markers to describe the location of a gene involved in a phenotype, followed by efforts to identify and isolate the gene using this starting information. Using this approach, a large number of human disease genes have been identified (Gusella *et al.*, 1983; Reeders *et al.*, 1985; Sherrington *et al.*, 1988).

Candidate Gene Approach. The candidate gene approach has been used to identify genes causing genetic disorders and genes associated with quantitative traits. It is based on associations between the phenotypes of interest and polymorphisms in genes known to be involved in the biochemical process being studied. While the candidate gene approach appears to be the logical approach, in general we do not possess the prior knowledge required to fully utilize this technology. At least in humans, the association between candidate genes and body mass index has been disappointing. No association has been found between this index and molecular markers of the glucose transporter-1, glucose transporter-4, insulin, insulin receptor, and glucocorticoid receptor (Weaver *et al.*, 1992a,b). However, associations between obesity and body fat levels with apolipoprotein B (Rajput-Williams *et al.*, 1988; Saha *et al.*, 1993), red blood cell acid phosphatase (Lucarini *et al.*, 1990), low density lipoprotein receptor (Zee *et al.*, 1992), apolipoprotein D (Vijayahavan *et al.*, 1994) and dopamine D₂ receptor have been suggested. Bouchard (1995) noted that a *Dra*I polymorphic restriction site in the alpha-2- adrenoreceptor gene was associated and linked with

subcutaneous upper body fat in women, independent of the overall level of fatness. Clearly, such a marker in meat animals has enormous potential implications for modifying fat distribution, particularly in the low value shoulder area. The limited number of published molecular marker studies suggests that there are at least several genes associated and/or linked with human obesity, and thus, potentially of value to other animal species of economic importance

Mouse Genetic Models of Adiposity. Mouse genetic models to date appear to be more informative than human examples from an animal production perspective. Also five mouse mutations causing obesity are encoded on five different mouse chromosomes (Bouchard, 1995). These mouse obesity genes are in coding regions that have human homologous equivalents. The *agouti* locus on mouse chromosome 2 regulates coat colour pigmentation. Several alleles at the *agouti* locus (A^y , A^{vy} etc) shift the relative proportions of black and yellow pigment of the hair of the animal (Yen *et al.*, 1994; Kwon *et al.*, 1994). Pleiotropic effects of the dominant *agouti* mutation are associated with obesity and increased susceptibility to diabetes. The *agouti* gene has been cloned and encodes for a 131 amino acid proteins. The gene is normally expressed in neonatal skin (Bultman *et al.*, 1992). However, the dominant yellow (A^y) mutation is associated with overexpression in a multitude of adult tissues (Yen *et al.*, 1994; Bultman *et al.*, 1992). The overexpression of the *agouti* protein interferes with the binding of the alpha-melanocyte-stimulating hormone to its receptor in the hair follicle, thus blocking the activation of adenylyl cyclase (Lu *et al.*, 1994). Another mouse obesity gene has also been recently cloned, the *ob* gene, which encodes a 4.5 kb adipose tissue mRNA with a 167 amino acid open reading frame. A nonsense mutation in codon 105 of the mouse gene is observed in the severely obese *ob/ob* line, and expresses a 20-fold increase in adipose tissue *ob* mRNA, while a second mutant strain does not exhibit any evidence for expression of the gene (Zhang *et al.*, 1994). The gene product is thought to be associated with appetite regulation.

Mouse QTL Experiments. Fisher *et al.* (1993) have recently identified new mouse genes for polygenic obesity. This was accomplished by using a backcross design between the strains *Mus spretus* and C57BL/6J; the resultant strain is termed the BSB mouse. The BSB strain exhibits a wide range of carcass lipid content ranging from 1-50%. On the basis of a QTL approach with a large number of markers, a number of informative loci have been located and have been termed *Mob-1 to 4* (Warden *et al.*, 1993; Bouchard, 1995). In another experiment, a mouse polygenic model of differential susceptibility has been developed by crossing the dietary lipid sensitive strain (AKR/J) with a resistant strain (SWR/J). After 12 weeks of feeding a high fat diet, the AKR/J strain had six times more fat than the resistant SWR/J STRAIN. F2 animals and back cross data were used and to date three QTLs have been identified, termed *Do1*, *Do2* and *Do3*. All are related to the level of adiposity and in the case of *Do2* it was related to mesenteric fat (West *et al.*, 1994a,b).

Meat producing animals. The leads given by the human and mouse genotyping efforts provide the farm animal geneticist with encouraging leads with respect to a number of QTL experiments which have been initiated in a number of species, including the bovine, porcine

and ovine in a number of laboratories around the world. Clearly the enormous collaborative effort in the human genome project has provided the basis for the genetic maps, which have been generated for farm animal species in the last 2 to 3 years. These comprehensive linkage maps and linkage studies, using a large number of markers covering the entire genome are likely to be helpful in elucidating the physiological basis and ultimately the regulation of fat deposition in farm animals. In the recently published gene map for the pig (Andersson *et al.*, 1994), a chromosome has been identified carrying genes (or quantitative trait loci) for backfat depth. They were fatness genes rather than leanness genes, because they came from the wild pig used to cross with the Large White in the experiment. Rothschild *et al.* (1995) have also identified markers in pigs associated with backfat depth.

CONCLUSION

The search for genes which are linked to animal growth and fat deposition and the molecular variants of these genes still remains a major undertaking. The task will be made more complex when researchers examine more complex issues such as gene-environment, gene-nutrient intake and gene-gene interactions. Many clues are likely to be derived from the human and mouse research fields. Clearly, the next decade will require the total integration of the disciplines of meat science, quantitative genetics and molecular genetics if any progress is to be made in developing farm animals which meet tight market specifications all of the time. Results from transgenic models will play an important role in revealing gene function and regulation of adiposity. The gene/QTL mapping approaches are likely to lead to discoveries of new genes, and advances in molecular biology are progressing at a rapid pace. In addition, the tools for the discovery and analysis of new genes continue to improve. The detailed analysis of gene structure and regulation of genes will provide a new impetus for the use new techniques for the production of transgenic livestock. Statistical methods continue to improve, which provides the quantitative geneticists with improved tools for the development of breeding programs to exploit QTLs, new genes and transgenic technologies.

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