

Physiology, Affecting Factors and Strategies for Control of Pig Meat Intramuscular Fat

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Abstract: Intramuscular fat (IMF) content is an important determinant of quality characteristics such as tenderness, juiciness and flavour, and of its acceptability by consumers. Research has therefore focused on improving meat quality by optimizing IMF. The present review describes evidence from studies of physiology, biochemistry, cell biology, genetics, candidate genes and nutritional regulation as well as animal trials relating to pig meat IMF and meat quality. Recent evidence suggests that breed and marker-assisted selection, candidate genes and nutritional regulation are the most promising strategies for improving IMF content. Additionally, some important aspects of IMF content research and related patents are also discussed.

Keywords: Intramuscular fat, pig meat quality, fatty acid metabolism, adipocyte differentiation, quantitative trait loci, dietary nutrition.

INTRODUCTION

Meat quality is an essential trait in meat-producing animals, especially pigs. With progress in breed selection and nutritional or non-nutritional manipulations, porcine growth performance has improved significantly and the amount of meat production has correspondingly increased. Conversely, however, pig meat quality has decreased. How to improve quality while maintaining or increasing the amount of pig meat production is therefore an ongoing challenge.

Meat quality describes the attractiveness of meat to consumers, which includes color, tenderness, water holding capacity, marbling and flavor. Due to increased consumer awareness with respect to eating quality and nutritional aspects of meat, qualitative research on meat is becoming more important. Studies have shown that intramuscular fat (IMF) content is one of the most important traits influencing eating quality characteristics [1]. Consequently, research on IMF deposition in the muscles of pigs and other meat-producing animals is currently one of the most important fields of study in meat quality science.

Researchers investigating genetic variation related to IMF deposition in pigs have identified certain quantitative trait loci (QTL), and the locations of these on porcine chromosomes have been posited [2,3]. Some functional or candidate genes have been identified and genetic polymorphisms have also been revealed [4,5]. However, IMF deposition is a complicated physiological and biochemical process which is regulated by metabolic enzymes and controlled by functional genes in the growth and development process of muscle and adipose tissues. Important foci in future research, therefore, include the molecular bases and mechanisms of IMF deposition and gene expression patterns and interactions in the deposition process. The present

review focuses on IMF physiology, IMF and pig meat quality, factors affecting IMF content of pig meat and strategies for improving IMF content.

PHYSIOLOGY OF IMF DEPOSITION

IMF

IMF refers to the chemically extractable fat in a muscle sample, predominantly from adipocytes (extramyocellular lipids, EMCLs) and myocytes (intramyocellular lipids, IMCLs). Morphologically, IMF is the total lipid associated with all cells present in a meat sample, excluding adipocytes from the intermuscular fat (fat surrounding the muscle tissue) depot Fig. (1). IMCLs localize within muscle fiber cytoplasm and EMCLs localize in adipocytes interlaced between muscle fibers Fig. (2). Chemically, these lipids can be subdivided into phospholipids, triacylglycerols (TG), mono- and diacylglycerols, cholesterol and cholesteryl esters, and free fatty acids. Phospholipids and TG are the major constituents of IMF. Phospholipids are the main

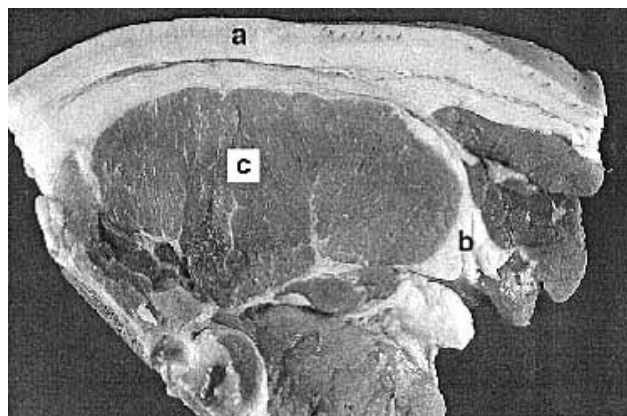


Fig. (1). Image of pig muscle and surrounding tissue showing (A) subcutaneous fat, (B) intermuscular fat surrounding the muscle tissue and (C) intramuscular fat between muscle fibres within the muscle tissue.

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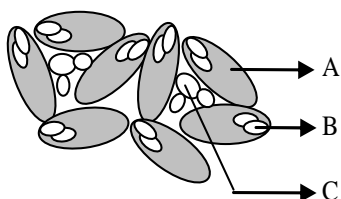


Fig. (2). Figure illustrating differences between intramyocellular lipids (B) and extramyocellular lipids (C) within and between muscle fibers (A).

constituents of cellular membranes and their contribution to IMF content in pigs is nearly constant within similar muscles but is variable between muscles. The proportion of phospholipids increases from white glycolytic to red oxidative muscle types [6]. Generally, an increase in IMF content is mainly due to an increase in TG content, as substantiated in studies with pigs [7,8]. Consequently, TG metabolism in muscle tissue should be a subject for identification of genes involved in genetic variation in IMF deposition.

Triacylglycerol Content in Muscle Fibres

Intramuscular TG are a major form of energy storage and represent a significant fuel for cellular metabolism [9]. In muscle tissue, TG are stored in adipocytes and myocytes, EMCLs and IMCLs respectively, existing in two fat compartments with similar fatty acid and triacylglycerol compositions. It has been reevaluated that IMCLs can lead to insulin resistance and be as a substrate in human endurance exercise [10,11], and their decrease associated with two PAT family proteins, adipocyte differentiation-related protein (ADRP) and tail-interacting protein (TIP47) [12]. But little is known about the roles of each EMCLs and IMCLs depot in the variation of IMF content.

It is well documented that oxidative (type I) muscle fibres contain considerably more intracellular TG than glycolytic (type IIa and IIb) fibres in muscle tissue [13]. The former occur in higher concentration in red oxidative fibers, the latter in white glycolytic fibres. Compared with the 'white' loin muscle *longissimu*, the *psoas major*, like many but not all 'red' muscles, is significantly more tender, suggesting that marbling fat is a marker for muscle fibre type and associated metabolic differences. Studies on the muscles of the calf have revealed that the highest IMF contents have been found in the medial part of the soleus muscle and lower contents in the tibialis anterior and posterior and the gastrocnemius muscle [14,15]. This matches the different fiber-type distribution of these muscles. The soleus muscle is a more oxidative muscle relying more on fat oxidation than the tibialis and gastrocnemius muscles. Likewise, the soleus muscle has a high IMF content, whereas the gastrocnemius muscle has a lower content of type I fibers. IMF content is dependant on the fiber-type composition of muscles, with oxidative muscle groups being characterized by a high IMF content. However, the TG content in muscles is related only to a minor extent to differences in muscle fibre type composition, mainly due to variation in the accumulation of adipocytes between muscle fibres [16]. Studies in rats and humans have shown that TG content in different metabolic types of muscle is associated with obese, insulin-resistant and type 2 diabetes [17-19]. However, no correlation has

been found in pigs between TG content and fibre composition of muscles of different metabolic types. On the other hand, a recent study has shown a positive correlation between mRNA expression of MyHC I, IIa, IIx in longissimus muscle and IMF content, but the relation between MyHC IIb mRNA expression and IMF content was dramatically negative [20].

IMF content can therefore be enhanced by optimizing TG content in adipocytes and myocytes of muscles and by optimizing the number of intramuscular adipocytes. Obviously, combinations of these opportunities should provide the best results.

Fatty Acid Metabolism in Muscle Tissue

Plasma lipid concentrations play a role in determining the rate of uptake of free fatty acids (FFA) into muscle tissues, circulating FFA concentrations are usually elevated in obese persons and animals, which coupled with reduced lipid oxidation resulting in excessive intramyocellular lipid deposition. Consequently, the excess muscle FFAs are either stored in lipid droplets or converted to various signaling molecules. This FFA conversion is predominately due to increased availability of fatty-acyl-CoA substrates for enzymes involved in synthesis of sphingolipids, eicosanoids, phospholipids, etc, and results in abnormal concentrations of these respective molecules, which may play a significant role in lipid-mediated insulin desensitization. In order for lipids to be used as fuel by skeletal muscle, FFAs must be taken up and converted intracellularly to long-chain fatty acyl-CoAs, imported into the mitochondria by carnitine acyltransferases, and subjected to β -oxidation Fig. (3) [21]. Fatty acid metabolism in intramuscular adipocytes is similar to that in myocytes.

The uptake of fatty acids by intramuscular adipocytes and myocytes in muscle tissue is facilitated by the enzyme lipoprotein lipase (LPL) located in capillary endothelial cells, which binds and hydrolyses lipoprotein TG - a rate-limiting step in fatty acid uptake [22,23]. Present studies have shown that fatty acids are translocated across the endomysial and sarcolemmal membranes and taken up by intramuscular adipocytes or myocytes in muscle tissue. Fatty acids can be translocated across these membranes by a simple diffusion mechanism or facilitated by membrane-associated proteins such as plasma membrane fatty acid-binding protein (FABP), fatty acid translocase (FAT) and fatty acid transporter protein (FATP) [24-29]. This transporter is key to regulating the increase in the rate of fatty acid metabolism in skeletal muscle. Although simple diffusion of fatty acids may occur, the major fraction of cellular fatty acid uptake is protein-facilitated under physiological conditions. FATP-mediated fatty acid uptake may cause intramuscular lipid accumulation leading to insulin resistance in muscle if the fatty acids are not oxidized. FATP-mediated fatty acid uptake may be driven by intrinsic acyl-CoA synthase activity [28,29]. However, the process of fatty acid uptake is not yet fully understood.

Intracellular fatty acids in intramuscular adipocytes are bound by fatty acid-binding proteins (FABPs), which are considered to be the-most important carriers for intracellular fatty acids. FABPs facilitate the transport of fatty acids from

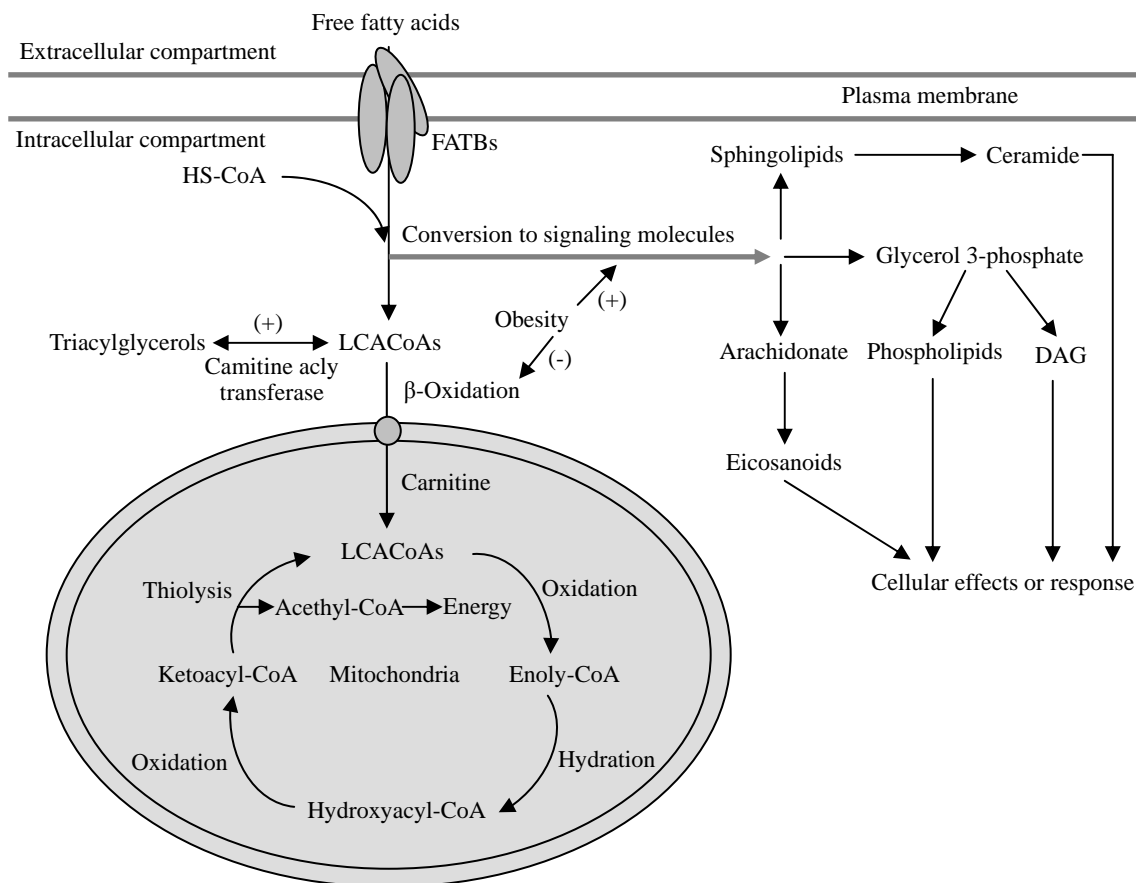


Fig. (3). Metabolism of free fatty acids to long-chain fatty acyl-CoAs (LCACoAs). LCACoAs can either be used for energy production through β -oxidation or undergo conversion to various signaling molecules, such as ceramide and diacylglycerol. Cited from Corcoran MP *et al.* (2007).

plasma membrane to the sites of fatty acid oxidation or esterification into TG or phospholipids. Moreover, FABPs may also be involved in bidirectional transport of fatty acids in intramuscular adipocytes [30, 31]. In this respect the interaction between hormone-sensitive lipase (HSL) and adipocyte-specific FABP (A-FABP) may be of significance. Recent studies have shown that the mobilization of intramuscular fatty acids in muscle tissue is regulated by HSL, an enzyme known to be rate limiting for intracellular TG hydrolysis in adipose tissue and found in all muscle fibre types [32]. The content of HSL varies between fibre types, being higher in oxidative fibres than in glycolytic fibres [33]. The signalling pathway in muscle by which HSL activity may be stimulated is protein kinase C (PKC) via extracellular signal-regulated kinase (ERK) [34, 35]. Other functions of FABPs may include protection of the cell from the deleterious effect of high concentrations of intracellular free fatty acids, and modulation of the action of (long-chain) fatty acids and other ligands, thereby influencing enzymes, membranes, receptors, ion channels and genes [25, 36-39].

Intramuscular Adipocyte Differentiation

It is well documented that adipocytes are highly specialized cells serving the crucial function of storage, metabolism and release of lipids, although the pattern and regulation of normal adipocyte growth and differentiation remain incompletely understood. Adipocytes are terminally

differentiated cells that develop from multipotent stem cells of mesodermal origin that also give rise to myocytes and chondrocytes. In animals and humans, the potential to generate new fat cells continues throughout the lifespan, and the reduction of adipocyte number may occur by adipocyte apoptosis and possibly dedifferentiation.

Previous studies mainly focused on adipose adipocyte differentiation and have demonstrated that the differentiation is influenced by a large number of protein factors. Some of the proteins regulating early differentiation of adipose adipocytes have been identified, which including CCAAT/enhancer binding protein (C/EBP), adipocyte determination and differentiation-dependent factor 1 (ADD1 or SREBP), C/EBP α undifferentiated protein (CUP or AP-2 α), peroxisome proliferator-activated receptor (PPAR), leptin, adipsin, and monocyte chemoattractant protein-1 (MCP-1) [40-44]. During adipose adipocyte differentiation, TG are synthesized from fatty acids synthesized *de novo* or supplied by the diet [45,46].

In recent years, researchers have paid particular attention to intramuscular adipocyte differentiation and TG storage, which play a dominant role in the process of IMF deposition. Present information indicates that porcine adipocyte number is achieved least in the intramuscular depot and adipocyte volume appears to be smallest in the intramuscular depots [47]. The intramuscular adipocytes are very late developing

and their number is very important to the total quantity of intramuscular lipid [48]. Intramuscular TG are stored both intramyocellularly, as droplets in the myofiber cytoplasm, and in adipocytes, interspersed between fiber fasciculi [49-51]. Due to the difficulty of separating intramuscular adipocytes from adjoining myofibers fewer studies have been devoted to intramuscular adipocytes compared to subcutaneous adipocytes, even though their number and size are the main determinants of TG and total lipid content variability in muscles of various species.

Evidence has accumulated that intramuscular adipocytes display differences in both metabolic and secretory functions from subcutaneous adipocytes. Intramuscular adipocytes display lower lipogenic activity compared to subcutaneous adipocytes. Recently studies have indicated that lipid metabolism and secretory function of intramuscular adipocytes differ from that of subcutaneous and perirenal adipocytes in growing pigs [52, 53]. Insulin-induced lipogenesis and lipolytic efficiency, gene expression and/or activities of enzymes involved in lipogenesis or lipolysis are much lower in intramuscular adipocytes than in adipocytes from other locations. In intramuscular adipocytic cells, the age-related increase in the ratio of mRNA levels of fatty acid synthesis to hormone-sensitive lipase parallels the enlargement of adipocyte diameters and the increase in lipid content in muscle during growth. Expression of genes coding for leptin, adiponectin, and IGF-I, as well as for various hormonal receptors, are lower in intramuscular adipocytes than in other adipocytes, whereas levels of TNF- α mRNA do not differ between sites. However, IGF-II mRNA levels are higher in intramuscular adipocytes than in other adipocytes [53,54], although the number of intramuscular adipocytes is less than subcutaneous adipocytes [55]. This indicates that IMF is not just an ectopic extension of other fat locations but displays specific biological features during growth. Recently, a method of co-culturing myotubes and preadipocytes from neonatal pig semitendinosus muscles has facilitated investigation of the relationship between adipocyte and muscle cell development within muscle [56]. To understand the relationship between intramuscular adipogenesis in the pig and the supply of fatty acids, Sanosaka *et al.* [57] established a clonal porcine intramuscular preadipocyte (PIP) line from the marbling muscle tissue of a female Duroc pig. The PIP cells exhibited adipogenic ability when cultured in differentiation medium for 8 days. Moreover, it was demonstrated that levels of caveolin-1 and actin gradually increased during adipose conversion in bovine intramuscular preadipocyte (BIP) cells, whereas a slight decrease for vimentin. Part of the vimentin was clearly distributed to caveolin-1-enriched membrane fractions in BIP cells, but actin was not. This suggested that a rearrangement of cytoskeletal proteins has a role in the intracellular accumulation of lipid droplets during adipogenesis of BIP cells [58]. Additionally, the respective collagen XVIII mRNA levels are upregulated during the differentiation of BIP cells. Although its function is unclear, these expression patterns indicate that type XVIII collagen may be associated with intramuscular adipocyte differentiation in cattle [59]. Another study indicated that no differences were detected in differentiation of bovine preadipocytes from intramuscular and subcutaneous treated with troglitazone [60].

Due to the limited research on differentiation and biology of intramuscular adipocytes and the relationship of which with IMF deposition, the search for the master regulatory factor in adipocyte differentiation continues, and identification may lead to understanding of those factors that switch on adipocyte determination and differentiation. Gene expression patterns related to intramuscular adipocyte differentiation and metabolism, and the underlying mechanisms regulating gene expression must therefore be clarified, as a necessary step in revealing the molecular bases and regulation mechanisms of IMF deposition and meat quality.

IMF AND PIG MEAT QUALITY

It is well documented that the three main components of meat eating quality are tenderness, juiciness and flavour. Tenderness is the most important, with tough meat being unacceptable. Variations in tenderness arise mainly through changes to the myofibrillar protein structure of muscle in the period between animal slaughter and meat consumption. If the carcass is refrigerated too rapidly immediately after slaughter, muscle fibres contract severely, so that the force required to shear the fibres after cooking increases dramatically. Changes to the myofibrillar proteins in muscle are also caused by proteolytic enzymes during ageing or conditioning. In recent years attention has focused on the possible links between tenderness and meat fat content. As fatness increases in the animal it does so in several body locations simultaneously, which could be important for tenderness. First, it accumulates in subcutaneous and intermuscular sites which could provide insulation for muscles against the effects of refrigeration as the carcass cools. Second, it accumulates in muscle (intramuscular or marbling fat) in the perimysial connective tissue. At high levels, when the amount of IMF exceeds 200mg/g muscle, it is possible that the muscle has a lower resistance to shearing because of the dilution of fibrous protein by soft fat. Also, fat cell expansion in the perimysial connective tissue forces muscle bundles apart, thus opening up the muscle structure [61].

It is now generally agreed that IMF content explains an important part of the genetic variation in the eating quality of pig meat [7, 62]. An IMF content below the recommended optimum range of 2.5-3% diminishes eating quality whereas a higher IMF content does not further improve this parameter and will have adverse effects on consumer acceptability due to increased visibility of fat in the meat [63].

In the early 1990s, IMF content was already well below the desired range of 2.5-3% in pigs produced in Europe [63,64] but not in pigs produced in the USA [65] and other countries. In China, IMF content is above 5% in traditionally produced local pigs [20, 66, 67]. However, IMF content in pigs has been decreasing as a result of unfavourable genetic selection for higher lean meat content and reduced backfat thickness. For example, each percentage improvement in carcass lean meat has been accompanied by a reduction in IMF content of 0.07% [68]. Particularly in China, because of the high backfat thickness and low lean meat of Chinese local pig breeds, in the last 20 years the selection for lower backfat thickness and higher lean meat has led to a significant decrease in IMF content, and has subsequently resulted in a severe reduction in meat quality. Consequently,

strategies to improve IMF content without reducing pig meat quality traits are essential for pork production.

FACTORS AFFECTING IMF CONTENT OF PIG MEAT

Genetic Control of IMF Content

Genetic Correlations with Meat Quality

Present evidence indicates that genetic correlations exist between IMF content and porcine carcass traits, and between IMF content and meat quality. Differences in body fat distribution between species or within species are genetically determined. Pigs present genetic correlations between IMF content and backfat thickness and differences in development of IMF relative to subcutaneous fat between genetically different breeds [69]. Genetic correlation between IMF content and carcass traits are showed in Table 1 [70, 71]. However, IMF content has been reported to be genetically correlated to various meat quality traits such as drip loss, water holding capacity, cooking loss, reflectance, tenderness, juiciness, overall acceptability, marbling, flavour and firmness of the meat. Some of these correlations are showed in Table 2 [70, 72-76]. Although for other meat quality traits these genetic correlations are only marginal, they do indicate that selection for increased IMF content will affect meat quality traits as well.

These correlations indicate that selection for increased IMF content will have adverse effects on the efficiency of selection on carcass traits. However, the range in size of the genetic correlations indicates that substantial genetic variation in IMF content is independent of these main carcass traits. In other words, selection for increased IMF content is feasible especially in situations where improvement in meat quality rather than carcass traits would be economically more beneficial.

Quantitative Trait Loci

The genome scan approach uses large numbers of available genetic markers and linkage maps to identify loci affecting quantitative traits (QTL). Recent technological advances such as Microarray-based analysis of single nucleotide polymorphisms (SNPs) have led to high-density QTL maps and more accurate determination of the responsible loci [77, 78]. With respect to IMF content several total

or partial genome scans have been performed in different pig populations (Table 3).

In a Meishan×Large White and Landrace F2 crossbred pig population initial autosomal analysis revealed suggestive QTL affecting IMF content on chromosomes 2, 4 and 6 (line cross model) and 4 and 7 (half-sib model) [2]. Adding a single marker to chromosomes 4 (A-FABP) and 6 (H-FABP) in the line cross analysis revealed only a suggestive QTL affecting IMF content for chromosome 6 but not for chromosome 4 [79]. In Large White × Meishan cross pigs there was a suggestive QTL for IMF on chromosome 4 and the Meishan's QTL increased the IMF content [80]. Moreover, another seven additional markers to chromosome 4 removed the 'half-sib' QTL whereas the suggestive QTL affecting IMF content under line cross assumptions was regained [81]. However, other eleven microsatellite markers or candidate genes localized on porcine chromosome 4, affecting IMF content, backfat thickness and other traits [82].

Autosomal genome scans based on line cross assumptions and accounting for imprinting identified two significant QTL affecting IMF content on chromosome 6, one maternally and one paternally expressed [83]. Moreover, a significant QTL affecting IMF content was identified on chromosome 6 in a Duroc× Norwegian Landrace×Large White population [84], in an Iberian×Landrace population [85], and in an Illinois Meishan× Yorkshire swine [86] as well as in an outbred-line pig cross commercially used in Norway [87]. Furthermore, fine mapping using combined linkage and linkage disequilibrium mapping confirmed QTL for IMF on porcine chromosome 6 within a 8.7-cM confidence interval in a commercial Norwegian slaughter pig cross [88]. Another fine mapping of porcine chromosome 6 QTL using SNPs in an Iberian × Landrace intercross revealed the presence of two QTL on SSC6. One, at position 60-100 cM, affected backfat thickness. The other more significant one maps in a narrow region (130-132 cM) and affected backfat thickness, IMF and eye muscle area [89]. Recently, Arnyasi *et al.* mapped the QTL region on porcine chromosome 6, confirming that the small heterodimer partner (SHP) and H-FABP genes affected IMF. Their map showed no recombination between SHP and H-FABP, which had been previously mapped to the same QTL region. H-FABP genotypes were confirmed to be significantly asso-

Table 1. Genetic Correlation Between IMF Content and Carcass Traits

| | Carcass Fatness | Carcass Leanness |
|-------------|-------------------------|------------------------------|
| IMF content | 0.44 - 0.6, average 0.3 | -0.55 - -0.07, average -0.34 |

Table 2. Genetic Correlation Between IMF Content and some Meat Quality Traits

| | Overall Acceptability | Firmness | Tenderness | Color | Fiber Type | Fiber Cross-Sectional Area |
|-------------|-----------------------|----------|------------|-------|--------------|----------------------------|
| IMF content | 0.61 | 0.31 | -0.09 | 0.43 | -0.05 - 0.06 | 0.68 |

Table 3. Main QTL for IMF Content in Various Experimental Crosses of Pigs

| Pig Population | Chromosomes Scanned | QTL Affecting IMF | Reference |
|---|---------------------|-------------------|-----------|
| Meishan×Large White or Landrace | | 4 | [2],[79] |
| Large White×Meishan | | 4 | [80] |
| Duroc× Norwegian Landrace×Large White | 1-18 | 6 | [84] |
| Iberian×Landrace | 1-18 | 6, 130-132 Cm | [85],[89] |
| Meishan×Yorkshire | 4,6,7 | 6 | [86] |
| Commercial Norwegian outbred-line cross | 1-18 | 6, 8.7 cM | [87],[88] |
| Meishan× Large White | 1-18 | 7 | [92] |
| Meishan × Duroc | 1-18 | 7 | [93] |
| <i>Sus scrofa</i> | 1-18 | 7, 17.1 cM | [94] |
| Meishan | 1-18 | 7 | [95] |
| Berkshire × Large White | 1-18 | 1 | [97] |
| | 1-18 | X | [98] |
| Duroc × Landrace | 1-18, X | 1, 6 cM | [3],[99] |
| | 1-18 | 13 | [3] |
| | 1-18 | 15 | [3] |
| | | 17, 32-39 cM | [99] |
| Pietran | | 8 | [101] |

ciated with IMF in pigs [90]. Also on pig chromosome 6, the SW71 microsatellite was located in the region corresponding to several QTL, such as those for IMF content and for body weight. The SW71 region contains eight genes and one putative gene [91]. This shows that the genomic organization of pig chromosome 6, including the gene order surrounding SW71, provides important information for comparative mapping. Moreover, the genes revealed in this study may be positional candidate genes associated with QTL on chromosome 6 that affect fat deposition in pigs.

In another Meishan×Large White crossbred pig population, a significant QTL affecting IMF content has been reported on chromosome 7 and also in a Meishan x Duroc F2 resource population, with the genome scan showing evidence for significant QTL affecting IMF content on SSC 7 [92,93]. Moreover, Soto *et al.* identified 34 microsatellite markers and 14 STSs in the 17.1-cM IMF QTL region, mapped previously on *Sus scrofa* chromosome 7 (SSC7). This indicates that IMF QTL had been fine-mapped to 12.6 cM between SJ169 and MM70 at the 0.1% chromosome-wise significance level. The SJ169-MM70 interval spans were approximately 3.0 Mb and contained at least 12 genes [94]. The detection of linked and pleiotropic QTLs influencing carcass composition traits on chromosome 7 of Meishan pigs revealed that Meishan alleles decreased carcass composition trait values except IMF, which offers an opportunity for marker-assisted selection to improve meat quality with maintenance of carcass composition based on Meishan alleles [95]. In a purebred Duroc population selected for meat production and meat quality traits over 7 generations, the QTL analysis for a full-sib family population was examined with the multi-generation pedigree structure of the population. The QTL was found at the 70 cM position for the loin eye muscle area, and at the 0 cM position for the pork

color standard, but no significant QTL for IMF were detected on SSC 7 [96].

On the other hand, Malek *et al.* identified significant QTL affecting total lipid content of muscle and marbling close to each other on chromosome 1 in a Berkshire × Large White crossbred population [97]. Also in this pig population a QTL affecting IMF content was reported for chromosome X [98]. A genome scanning on F2 Duroc × Landrace pigs showed that the QTL at chromosome 1 position 6 cM presented significant evidence for IMF, and the region on chromosome 17 (32-39 cM) was associated with both IMF and loin eye area [99]. Subject to verification, these QTL for IMF should be useful in commercial production to improve pig meat quality.

Recently, using an informative SNP for the Iberian × Landrace intercross detected in intron 12, a linkage map was constructed. The QTL for IMF was confirmed with high significance, and its position was narrowed down to an interval of 4 cM. The region was defined by markers PDE4B and SW1881, and 5 genes were selected as positional and/or functional candidates related to the QTL [100]. In another study, QTL for chemical and physical body composition in pigs were identified in a three-generation full-sib population, developed by crossing Pietrain sires with a commercial dam line. Novel QTL for lipid content and lipid accretion were detected on SSC9. Another QTL for lipid accretion was found on SSC8, closely associated with a QTL for IMF content [101]. In an experimental F2 Duroc × Large White population, QTL was detected first by segregation analysis, then by QTL mapping using additional molecular information. Evidence was found for three QTL significant at the chromosome wide level. Two QTL, located on chromosomes 13 and 15, showed a high IMF Duroc

recessive allele with an overall effect slightly lower than that expected from segregation analysis. The third QTL was located on chromosome 1, with a dominant Large White allele inducing high IMF content [3]. This result suggested that the favourable alleles inducing high IMF content were not fixed, and that improving IMF by fixing favourable alleles using markers can then be applied both in Duroc and LW populations. With QTL affecting fatty acid composition, combining an increase of IMF content enhancing monounsaturated fatty acid percentage would be of great interest.

From these studies, other chromosomes may also contain QTL for IMF in the respective pig populations. Moreover, results from QTL studies may vary according to the prior assumptions in the analyses as well as the genetic background of the pigs under investigation. Despite these reservations, it is worth noting that several studies report chromosome 6 to contain one or two QTL that affect genetic variation of IMF content. These QTL are located in a similar region on the long arm of chromosome 6. The QTL region in some studies is still too large to identify positional candidate genes; however, a joint analysis of all available material for IMF content in a similar approach to that taken for backfat thickness may prove these QTL to be genuine and may significantly reduce the QTL region. Therefore, it should be noted that many of the aforementioned QTL affecting IMF content are in the proximity of QTL affecting backfat thickness, and eventually may prove to be the same genes. Fine-mapping of QTL affecting IMF on porcine chromosome is therefore important, with identification of single gene positions in the QTL region as well as investigation of QTL affecting IMF on chromosome in other breeds of pigs, especially some native traditional local breeds.

Candidate Genes

After showing the presence of QTL affecting IMF in porcine chromosomes, researchers have turned their attention to candidate or functional genes affecting IMF content. In recent years, some candidate genes affecting IMF content have been investigated on the basis of gene structure, SNPs and gene expression in tissues. Various candidate genes in livestock have been successfully identified as having linkage associations (ESR, A-FABP, H-FABP, FUT1, MC4R, MYOG, PRLR) or even causative relationships (GDF-8, Ryr-1, KIT, MSHR) with various traits such as (re)production, disease resistance, meat quality and coat color [102]. Seven candidate genes have been reported to have associations with IMF content: H-FABP, A-FABP, ADRP, LERP, MC4R, ADD1(SREBP1), MYOD, and there are possibly others. This far, most attention has been given to the H-FABP and A-FABP genes.

The H-FABP gene encoding muscle-specific fatty acid-binding protein has been significantly associated with genetic variation in IMF content, backfat thickness and growth in purebred Duroc pigs [103]. The H-FABP gene resides on porcine chromosome 6 within or close to the QTL region affecting IMF content identified in several crossbred pig populations [2,84,104]. Subsequent analysis has shown that H-FABP genotypes have a significant effect on IMF content in the Duroc×Norwegian Landrace×Large White crossbred population [84], in a Iberian×Landrace crossbred population [105], in four Chinese local pig breeds (Bamei

pig, Hanjiang Black, Hanzhong White, and wild pigs) [66] and in a Meishan×Large White and Landrace population [79]. Gene polymorphism analysis showed that polymorphism of H-FABP was associated with IMF content in Large White and Landrace breeds [106] and in Meishan, Setai and Duroc×Landrace×Yorkshire pig populations [107]. On the other hand, others found no significant influence of the H-FABP gene on IMF content in Austrian Piétrain, Large White and Landrace breeding populations or Australian commercial pigs [108].

To validate the role of A-FABP and H-FABP in IMF accretion, Gerbens *et al.* [109] investigated A-FABP and H-FABP polymorphisms, mRNA, and protein expression levels of both FABP genes and IMF content. Significant differences were observed in H-FABP mRNA levels but not protein expression levels in H-FABP genotype classes [109]. Moreover, H-FABP mRNA but not protein expression levels were significantly related to IMF content. Considering gene expression in tissues, H-FABP was expressed in subcutaneous and intramuscular porcine adipocytes, and H-FABP at a lower level in intramuscular adipocytes than in subcutaneous adipocytes [110]. Also, Li *et al.* [111] showed that expression of H-FABP mRNA in adipose tissue was higher than in skeletal muscle. Furthermore, levels of H-FABP mRNA reached a maximum in adipose tissue from neonates, with no further increase in the adult. Also, H-FABP mRNA was induced during adipogenic differentiation of stromal-vascular cells derived from adipose tissue and skeletal muscle. This suggests that H-FABP may play a role in adipose tissue development and function in the pig.

A number of genes have been shown to be closely linked to the H-FABP gene in humans and mice. One particular gene should be highlighted: the leptin receptor gene (LEPR), which mediates the effects of leptin, the major hormonal controller of long-term energy balance [112]. The H-FABP and LEPR genes are located about 20 cM apart on porcine chromosome 6 with LEPR telomeric to the H-FABP gene. Candidate gene analysis showed that the LEPR gene is also significantly associated with IMF content [89,105]. Of course, correlated traits may result in similar effects in candidate gene analyses. The H-FABP gene also affects backfat thickness in the purebred Duroc pigs but these effects have been shown to be independent [103]. Similar results were found by Ovilo *et al.* for the H-FABP gene but not for the LEPR gene [105]. Grindflek *et al.* found no QTL for backfat thickness on chromosome 6, also indicating an independent association with IMF content [84].

The A-FABP gene encodes adipocyte-specific FABP and has been shown to be significantly associated with IMF content independently from backfat thickness in purebred Duroc pigs [113]. The A-FABP gene resides within a QTL region affecting IMF content on chromosome 4 in a Meishan × Large White and Landrace pig population [2] and other fatness traits in other pig populations [114-116]. However, the effect of A-FABP on fatness traits could not be substantiated in these populations [79] or in other Austrian pig breeds [108]. Neither do the results of QTL studies suggest a general QTL affecting IMF content on chromosome 4 [84, 97, 105]. In specific pig breeds, genes closely

linked to the A-FABP gene on chromosome 4 of the pig are most likely to be responsible for the effect on IMF content.

Similar to H-FABP, A-FABP is expressed in subcutaneous and intramuscular porcine adipocytes and at a lower level in intramuscular adipocytes than in subcutaneous adipocytes. However, a discrepancy has been observed between age-related changes in A-FABP content in isolated adipocytes and cell diameter or lipid content variations in tissues during growth [110]. For A-FABP genotype classes, no significant differences in mRNA and protein expression levels have been found. A significant relationship between A-FABP mRNA but not protein expression levels and IMF content has also been found [109].

Taking all data into account, the existence of significant associations between H-FABP and LEPR and IMF content in independent and distinct pig populations indicates that these or closely linked genes are responsible for part of the genetic variation in IMF content in particular pig breeds or populations. Data suggest that two QTL may reside in a similar region, one affecting IMF content and the other affecting backfat thickness. Variation of IMF content cannot be explained by differences in A-FABP and H-FABP mRNA and protein expression levels, although this may be due to limitations of the assays used and/or the inappropriateness of the time of sampling. Finally, results suggest that A-FABP and H-FABP expression are translationally rather than transcriptionally regulated. So far, a causal relationship with variation in IMF content has not been clearly shown for H-FABP, A-FABP or LEPR, but these gene variants may still exist in pigs.

In addition to the above genes, some others have recently been reported as presenting potential associations with IMF content. The transcription factor adipocyte determination and differentiation-dependent factor 1 (ADD1), also called sterol regulatory element-binding protein 1c (SREBP1), regulates the transcription of fatty acid synthase and plays a role in adipocyte differentiation [117]. Polymorphisms of ADD1 gene have been found associated with IMF content in Meishan, Suta and Duroc \times Landrace \times Yorkshire pigs [107]. Moreover, SREBF1 expression and genetic polymorphisms presented positive associations with IMF content in Erhualian and Suta pigs [118]. These results suggest that ADD1 (or SREBF1) could be used as a genetic marker to improve IMF content in pigs.

Adipose differentiation-related protein (ADRP) plays an important role in regulating lipid storage in various cells. Kim *et al.* [119] reported the ADRP gene as a candidate gene for IMF deposition and marbling traits in pigs. Polymorphisms of porcine melanocortin-4 receptor (MC4R) have been associated with IMF content in Meishan, Suta and Duroc \times Landrace \times Yorkshire pig populations [107]. Moreover, MC4R polymorphism correlated with lower levels of IMF content in Polish Landrace, and increased levels of IMF content in Polish Large White [120]. Another study showed that the level of lipoprotein lipase (LPL) and malic enzyme (ME) mRNA expression correlated with IMF development in Erhualian pigs. This suggests that the expression of ME and LPL mRNA may contribute to rapid deposition of IMF in Erhualian pigs [121].

Members of the porcine myogenic differentiation genes (MYOD) family play key roles in growth and muscle development and can therefore be considered candidate genes for IMF content and meat production traits. A recent study has shown that significant associations are observed between MYF5 gene and IMF content in full- and half-sibs of Large White and Landrace breeds, and highly significant differences in IMF content are observed between genotypes AA and AB of MYOD1 [122]. Another study has revealed that porcine liver-type fatty acid binding protein gene (L-FABP) is expressed in all tissues, but a transcript is most abundant in liver and small intestine. Comparative sequencing of four pig breeds revealed a C \rightarrow T SNP within exon 2. Analysis suggests that this polymorphism is associated with IMF content, indicating that the SNP is a potential molecular marker [123].

Carbonic anhydrase 3 (CA3) is a member of the carbonic anhydrase family, which plays an important role in various cell processes. Molecular characterization has shown that CA3 genomic DNA consists of seven exons and six introns, spans about 10.5 kb, and maps to porcine chromosome 4 q11 \rightarrow q14. Statistical analysis showed that CA3 gene polymorphism differed in Chinese indigenous and introduced commercial western pig breeds, and was associated with IMF content of pigs [124]. A study on the pig p160 co-activator family indicated that three transcript variants were porcine nuclear receptor co-activators 1 (NCOA1), with two in the porcine NCOA2 gene but none in NCOA3. Both NCOA1 transcript variant 2 and total NCOA1 expression levels were negatively correlated with IMF contents, while NCOA2 transcript variant 1 and NCOA3 were positively associated with IMF content in *Longissimus dorsi* muscle [125]. Interestingly, another study revealed that mitochondrial DNA polymorphisms were associated with IMF and protein content in an Iberian porcine line [126].

Despite the present data indicating that polymorphisms and expression levels of candidate genes are associated with IMF content of pigs, the causally significant or positive relationship between candidate genes and IMF content still requires extensive research on different genotypes and breeds of pigs. In particular, new candidate genes relating to IMF content need to be identified, and it will be essential to elucidate the interactions and tissue expression of candidate or functional genes and the regulatory mechanisms of gene networks in IMF deposition and muscle tissue development.

Nutritional Factors Affecting IMF Content

Dietary Nutritional Level and Sources

There is evidence that dietary nutritional level and sources can affect porcine IMF content. Feeding lysine-deficient diets at the end of the finishing period of pigs increases IMF content while having no effect on marbling scores in the longissimus muscle [127]. Moderate long-term feed restriction (low protein and energy intakes) resulted in a decreased lipogenic capacity of muscle adipocytes and decreased IMF content [128]. Dietary restriction (20% less protein and 7% less energy) of growing pigs resulted in the accumulation of significantly more IMF, and in the increased expression of genes involved in substrate (protein, glycogen, and lipid) turnover, in translation and mitochondrial

function, and in raising glycolytic and oxidative phosphorylation potentials in both red and white muscles [129]. Moreover, a lower feeding level (50g feed per kg of weight) increased the proportion of C18:3 (n-3) fatty acids and decreased monounsaturated fatty acids in porcine IMF to a greater extent than did a higher feeding level (70g feed per kg of weight). IMF from lower feeding level pigs had significantly higher proportions of C18:0, polyunsaturated fatty acids, and significantly lower monounsaturated fatty acids and C18:1 (n-9) proportions than higher feeding level pigs [130]. A reduced protein diet (RPD) is known to increase the level of intramuscular lipid in pig meat with a smaller effect on the amount of subcutaneous adipose tissue, which might be due to tissue-specific activation of the expression of lipogenic enzymes by the RPD. A recent report has indicated that an RPD significantly increased stearoyl-coenzyme A desaturase (SCD) protein expression and activity in muscle but not in subcutaneous adipose tissue. Moreover, there was a positive significant correlation between SCD protein expression and total fatty acids in muscle [131]. This suggests that an increase in intramuscular but not subcutaneous adipose tissue fatty acids under the influence of an RPD is related to tissue-specific activation of SCD expression. Furthermore, current studies on Chinese Wujin pigs have indicated that higher dietary digestible energy level or lower dietary protein level could increase porcine IMF content [132,133]. However, residual feed intake could decrease the growth performance of pigs without affecting IMF content and carcass composition [134,135].

In pigs given added rapeseed oil to their feed, the fatty acid composition of backfat and the triglyceride fraction of IMF became more unsaturated. Higher intake of monounsaturated fatty acids in the rapeseed oil affected the phospholipid fraction of the IMF, but not the proportion of saturated fatty acids [136]. The effects of four protein sources (soybean meal, sunflower meal, pea, and fish meal) on ileally digestible Lys:DE ratios in pig diets indicated that the protein sources had no effect on lean meat percentage, IMF content and meat quality. However, lowering the Lys:DE ratio increased crude fat and fatty tissue content and decreased protein and muscle content of the porcine body [137]. Additionally, pigs fed diets containing 1/3 yellow corn and 2/3 white corn had a greater percentage of IMF than pigs fed diets containing either yellow corn or white corn [138]. Another study showed that diets supplemented with selenium and vitamin E also resulted in an increase in porcine IMF content [139]. However, diets without synthetic amino acid supplementation increased porcine IMF content while reducing growth performance [140].

Long-Chain Polyunsaturated Fatty Acids

Long-Chain polyunsaturated fatty acids (LCPUFA) are often present at low levels in meat, especially those of the n-3 series which have particularly beneficial effects on health. In recent years, many workers have sought ways to change meat fatty acid composition, mainly through feeding plant or fish oil sources of PUFA. This dietary regimen can alter meat quality by providing a different mix of reactive ingredients which affect oxidative stability and flavour.

Pigs fed n-3 PUFA sources (equivalent linseed and fish oil) of diets resulted in the greatest eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA) proportions in longissimus thoracis, but the content of docosapentaenoic acid (DPA) was not affected [141]. On another study, pigs fed diets containing soyabean oil resulted in the deposition of n-6 and n-3 LCPUFA in carcass meat. The content of 18:1 fatty acids increased, but that of 16:0 and 18:0 fatty acids decreased during the finishing period [142]. Feeding the whole linseed diets increased alpha-linolenic acid, EPA and DHA concentration in pig longissimus thoracis. The changes in fatty acid composition resulted in marked changes to the n-6: n-3 and arachidonic: EPA ratios with enhancing the levels of n-3 fatty acids [143]. Moreover, the linseed diet increased the content of n-3 PUFA in pig muscle and adipose tissue, but DHA was not altered. The linseed diet produced a PUFA:saturated fatty acid ratio > or = 0.4 in all tissues [144]. This suggested that linseed (flaxseed) in swine diets is a valid method of improving the nutritional value of pork without deleteriously affecting organoleptic characteristics, oxidation, or color stability. Recently, Kawashima *et al.* discussed the method for producing phospholipids that contain LCPUFA as a constituent (LCPUFA-PL), lipid producing cells producing lipids that contain LCPUFA, by extracting TG-containing oil or fat from the lipid producing cells [145].

On the other hand, broiler chicks fed with dehydrated forages (good sources of alpha-linolenic acid, ALA) resulted in significant effects on meat fatty acid profile. Forages did not affect the linoleic acid and ALA contents in poultry meat, but the levels of n-3 LCPUFA (EPA, DPA and DHA) in breast meat were significantly higher [146]. Similar effects were observed in legume-based pasture diets feeding [147]. Diets supplemented with fish oil decreased the saturated and monoenoic fatty acids contents in chicken meat. The amount of PUFA increased, mainly as n-3 LCPUFA. However, levels of total n-6 LCPUFA resulted in slight changes, mostly in linoleic acid [148]. Similarly, supplying linseed oil in chicken diets clearly decreased the saturated and monounsaturated fatty acids (MUFA) contents and increased the amount of n-3 LCPUFA in meat [149]. Additionally, feeding high-oil corn in finishing beef cattle diets increased linoleic acid, arachidonic acid, the total PUFA, and total odd-chain fatty acid content, but decreased saturated fatty acid percentage of the longissimus. This indicated that high-oil corn could enhance intramuscular lipid deposition and increase unsaturation of fatty acids of the longissimus in beef cattle [150].

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is a mixture of isomers of linoleic acid that occur naturally in food. Many studies have shown that dietary intake of CLA (*cis*-9, *trans*-11 and/or *trans*-10, *cis*-12 isomers) changes porcine body composition and fatty acid profile in adipose tissue and muscle, increasing IMF deposition while decreasing subcutaneous fat and improving meat quality [151-157]. Moreover, the underlying mechanisms of CLA on subcutaneous adipose tissue and intramuscular adipose tissue have been to some extent elucidated. CLA (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers) increases the expression of PPAR γ

and A-FABP in muscle, indicating that it can induce the development of preadipocytes from stromal-vascular stem cells to promote IMF content. On the other hand, the increase in expression of glutamine-fructose aminotransferase (GFAT) mRNA indicates that the glucose supply of muscle cells is increased with the CLA diet, possibly sparing intramuscular fatty acid reserves [151]. Furthermore, CLA decreases the expression of adipocyte-specific genes as well as adipose precursor cell numbers and the accumulation of lipid in cultured subcutaneous adipose tissue stromal-vascular cells. Additionally, CLA (trans-10, cis-12 isomers) increases the expression of adipocyte-specific genes in intramuscular cultures, resulting in an increasing accumulation of lipid [158]. These data show that CLA decreases subcutaneous adipose tissue and increases intramuscular adipose tissue by different mechanisms of regulation of adipocyte-specific gene expression, which indicates that adipogenesis in intramuscular adipocytes differs from that in subcutaneous adipocytes.

Non-nutritional Factors Affecting IMF Content

During the 1990s, porcine somatotropin caused interest in growth performance and carcass characteristics of pigs. Reports showed that porcine somatotropin administration resulted in improved growth rate, increased carcass protein and water content, reduced carcass fat content and IMF content, increased unsaturated fatty acids and decreased fat cell diameter in backfat, without any effect on other meat quality traits [159-161]. Because of consumer safety concerns, however, porcine somatotropin administration was prohibited by the end of the decade.

Also in the early 1990s, a comparatively safe immune approach was developed for use in pigs and other animals, involving the subcutaneous or abdominal injection of anti-adipocyte plasma membrane protein polyclonal [162] or monoclonal [163] antibodies. Studies showed that polyclonal antibodies could significantly reduce porcine backfat and increase the percentage of lean meat, although IMF content was also reduced due to antibody non-specificity [162,164]. Monoclonal antibodies, however, displayed similar effects on porcine carcass composition, but, owing to their high specificity, had no effect on, or even increased, IMF content, and could depress subcutaneous adipocyte differentiation *in vitro* and *in vivo* as well as regulate porcine lipid metabolism [163,165-167]. Although much remains to be resolved before this method can be applied to pig production, the immune approach remains a promising strategy to reduce the percentage of backfat. Indeed, another of its potential applications may be in human obesity.

Environmental Factors Affecting IMF Content

Besides genetic and nutritional factors, environmental factors may affect IMF content. Maintaining pigs at a high environmental temperature (32°C) resulted in an increase in carcass length but had no effect on other carcass measurements or IMF [168]. However, rearing of free-range pigs outdoors during winter (average temperature 5°C) had no effect on intramuscular lipid content of the semiten-dinosus, but intramuscular lipid content was lower in the longissimus muscle and tended to be higher in the *rectus femoris* than in pigs reared in indoor housing (22°C) [169].

This indicates that environmental temperature has some effect on porcine IMF.

STRATEGIES IMPROVING IMF CONTENT OF PIG MEAT

Breed Selection

Different strategies can be used to improve IMF content of pig meat. The most obvious would be to take advantage of the positive genetic correlation between IMF content and body weight; that is, a higher weight at slaughter would result in increased IMF content and hence improved meat quality. However, this strategy results in a lower lean percentage of the carcass and would require a new conformation of the slaughter industry to higher carcass weights. The most promising strategy is based on the observation that IMF content can be improved by selection. Heritability estimates for IMF content indicate substantial genetic variation in this trait. The high heritability estimate indicates a considerable selection response in direct selection programmes. However, genetic correlations of IMF content with production and other meat quality traits and the accuracy of current methods to assess IMF content should also be taken into account.

Marker-assisted Selection

Another technique is the direct exploitation of the genes or genomic loci that control IMF content. The size of the genetic correlations between IMF content and production traits, as mentioned above, indicate that part of the genetic variation is independently inherited. In other words, these traits are partially controlled by different genes. Hence, traits may be treated independently when the respective genes that contribute to the genetic variation of IMF content but not of production traits are identified. Evidence for the existence of genes with a substantial effect on IMF content has been provided by segregation analysis in pigs. Upon identification, these polymorphic genes or genomic regions may be applied in breeding by eliminating detrimental allele(s) from the population, by marker-assisted selection (MAS), by gene-assisted selection (GAS) or by introgression of beneficial allele(s) in populations or breeds lacking these alleles. Actual application of QTL data in breeding schemes depends on the absence of genes that adversely affect other important breeding traits, such as average daily gain and backfat thickness, in the QTL region affecting IMF content. MAS can considerably increase the selection response, in particular for traits with a low heritability and/or for carcass and meat quality traits such as IMF content. GAS is an effective selection scheme to increase the genetic gain and the economic returns in pig breeding using the QTL-linked direct marker. To identify the genes and genomic loci responsible for genetic variation in IMF content two approaches can be employed: the genome scan and candidate gene analysis approach.

Recently, some genetic marks for IMF content or meat quality have been successfully selected. The genetic marker based upon the presence or absence of certain polymorphisms in the PRKAG3 gene was selected by Rothschild *et al.* in US20050208551 patent [170]. A leptin receptor gene as a genetic marker for IMF content and meat quality in pigs was selected by Kojima *et al.* using single nucleotide polymorphism method in EP0778518 patent [171]. Other

genetic marks based on single nucleotide polymorphism in FABP4 and CRH genes were also selected by Jiang *et al.* in WO2006128116 and WO2007109702 patents [172,173]. Genomic marker for meat tenderness is also studied by Bernard *et al.* in EP20060300943 and WO08031846 patents [174,175]. These aspects might provide for novel methods which may comprise marker-assisted selection or marker-assisted management to improve IMF and/or meat quality in pig.

Candidate Gene Approach

In addition to the positional candidate genes from the QTL regions affecting IMF content mentioned above, genes may also be candidates based on existing knowledge of physiological and biochemical processes, also known as the candidate gene approach. From a statistical genetic point of view, the candidate gene approach is a linkage disequilibrium mapping approach using linkage disequilibrium across families in a population. Benefits of the candidate gene approach are the relative directness and low costs. However, success largely depends on the amount and quality of prior information to identify candidate genes in the pig population under investigation.

In recent years, using the annealing control primer (ACP)-differential display RT-PCR method, Lee *et al.* selected a novel transcription repressor ICER gene, which might be a candidate gene that participates in intramuscular fat development [176]. In addition, The genetic markers in the calpain3 (CAPN3) and mitochondrial transcription factor A (TFAM) genes were studied by Barendse and Jiang *et al.* in WO2007053891 and US20070065843 patents [177,178]. Blowe discussed the genetic marker for certain polymorphisms in the pig follistatin gene [179]. Moreover, whole genome based genetic evaluation and selection process is also discussed by Raadsma in WO08025093 patent [180]. These research might provide methods facilitating candidate gene selection to improve IMF and/or meat quality in pig.

Nutritional Regulation

IMF deposition is a complicated physiological and biochemical process, which is regulated by metabolic enzymes and controlled by related functional genes in the growth and development process of muscle. Besides genetic control, based on evidence accumulated in recent years that dietary nutritional level can regulate tissue metabolism and gene expression, the process of IMF deposition can be regulated by nutritional state or dietary nutritional level. Consequently, with respect to improving IMF content in muscle tissue, dietary nutritional regulation is another significant approach. The process of IMF deposition needs a high energy supply in diets in order to satisfy the requirement for muscle development and to increase the content of IMF in muscle. Therefore, high-energy low-protein is the suitable dietary nutrition level for improving IMF content, although appropriate growth rate, better carcass composition and meat quality must also be taken into account. Other methods such as supplying CLA or plant oil in diets also provide effective approaches. Genes for plant fatty acid modifying enzymes are discussed by Cahoon *et al.* in US20080171860A1 patent [181].

Ultimately, the implementation of strategies to improve meat quality depends on the willingness of consumers to purchase and pay more for meat of better quality. To market meat of better eating quality, consumers need to be able to discriminate between different categories of eating quality, thus favouring meat grading, branding and labeling. Certainly, improvement of meat quality by optimizing IMF content will not only be appreciated by the consumer but will also be profitable to the meat industry.

CURRENT & FUTURE DEVELOPMENTS

It will be apparent that current data on IMF content of pig meat has been derived mainly from studies on physiologic, genetic, and nutritional factors affecting IMF deposition. However, extensive research is still required in each of these areas.

First of all, the search for the master regulatory factor in intramuscular adipocyte differentiation still continues and identification may lead to understanding of those factors that switch on intramuscular adipocyte determination and differentiation. This may give insight into the key factors that regulate intramuscular adipocyte differentiation and into the molecular mechanisms regulated by these factors, as well as the biology of intramuscular adipocyte metabolism, ultimately revealing the underlying mechanisms of IMF deposition. Adipogenesis regulates by secreted protein ccdc80 is discussed by Gimeno and Tremblay in US20080221057A1 patent [182].

Secondly, fine-mapping of QTL affecting IMF on porcine chromosomes and identifying the single gene positions in QTL region may further facilitate the accurate marker-assisted selection or gene-assisted selection in breeding. Continuous investigation of QTL affecting IMF on the chromosomes in other breeds of pigs, including some native traditional local breeds, might result in the discovery of some new QTL affecting IMF and may provide insight into the genetic variation of IMF content and meat quality traits.

Thirdly, analysis of gene expression profiles during the process of IMF deposition and the identification of new functional genes or expression sequence tags (ESTs) relating to IMF deposition will provide important information on gene expression in muscle tissue development and adipocyte differentiation. This should lead to a better understanding of the interactions and tissue expression of functional genes and the regulatory mechanisms of the gene networks controlling IMF deposition and muscle tissue development, which might facilitate the candidate gene approach in breeding. With current tools such as high expression cDNA microarray technology and computerized bioinformatics, the analysis of gene expression profiles and identification of new functional genes or ESTs are readily accomplished. This will result in a better understanding of the genomic regulation of tissue formation, improved knowledge of the genomes under selection, and may lead eventually to directed breed-specific changes. Woodward discussed in his patented work the identification of single nucleotide polymorphisms (SNPs) within the bovine gene encoding mitochondrial transcription factor A (TFAM) and their associations with economically relevant traits in beef production [183].

Furthermore, investigation of nutritional regulation pathways and mechanisms of IMF deposition will facilitate successful regulation to improve IMF content. In this respect, the considerable number of genes or proteins involved in fatty acid metabolism in muscle and adipose tissue should be a research focal point, since this may eventually lead to an understanding of the genes controlling the amount of fat deposited in muscle tissue. Neuroendocrine regulatory mechanisms of fatty acid metabolism in muscle should be taken into account as well, however, since nutritional regulation may be effected through neuroendocrine pathways to which some hormones or proteins have contributed. For example, insulin, leptin and RBP4 are involved in insulin resistance which is associated with increased TG content in skeletal muscle.

Therefore, because of the intricate processes involved in IMF deposition there are many facets to be considered, all of which must be examined carefully before practical implementation can be effected. Fatty acid can be efficiently screened by novel screening method is proposed by Ito *et al.* in US20080160033A1 Patent [184].

CONFLICT OF INTEREST

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