ANIMAL BIOTECHNOLOGY: PIG MUSCLE PART I, FACTORS AFFECTING MUSCLE FIBER TYPES IN PIGS

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Abstract Today, the study of muscle fibers is critical because they are responsible for the variation of growth performance and meat quality traits in farm animals. There are several factors that can contribute to alter the muscle fiber composition. Some of them genetically originate from individual, breed, gender, birth & slaughter weight and genetics, while others are environmental factors such as prenatal and postnatal nutrition, temperature and physical activity. Chiang Mai Veterinary Journal 2007;5(1):81-91.

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The mammalian skeletal muscle is composed of a heterogeneous collection of fiber types with polynucleated and elongated cells that can be classified according to their myosin heavy chain (MyHC) isoforms, contractile elements (microfilaments), energy metabolism, fiber color or cross-sectional area (CSA) as showed in table 1.1-3 In farm animals, the structural and functional diversity of skeletal muscle is represented by the variety of myosin isoforms. Understanding factors influencing muscle fibers will help to optimize the efficiency of the muscle growth and meat quality, which are of important concerns in animal production. In this review, the effects of both genetics and environments on the distribution of muscle fibers will be discussed with the emphasis on pigs.

Genetic factors

Muscle and individual

The composition of fiber types varies between anatomical muscles (Fig. 1). The distribution of pig muscles is unique and highly...
organized, in which deep muscles contain more type I fibers surrounded by an internal rosette of type Ila fibers and an external ring of type IIb fibers.\(^4\) Functionally, type I fibers are more involved in maintaining posture, while type IIb fibers are responsible for rapid movement.\(^5\) In commercial breeds, the proportions of type IIb and IIX fibers of *longissimus dorsi* and *psoas* muscle are highest compared with the other fibers as pointed out by many research groups.\(^6-10\) The same tendency was also noticed in *biceps femoris*, *quadriceps femoris*\(^6\) and *adductor*.\(^11\) On the other hand, examples of high degree of type I and Ila fibers can be seen in *rhomboideus* muscle.\(^9,10\)

Animals of the same breed reared in the same environment also show large variation in fiber type composition. An example is the variation within litter of Danish Landrace x Large White (LW) pigs, as described by Nissen et al. (2004).\(^12\) In this experiment, they slaughtered and grouped the animals by litter, at the same age, according to the body weight: heaviest, middle and lightest weight. It was found that, intra-litter growth performance varied largely as a result of differences in both the number and

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**Table 1.** Classification of skeletal muscle fiber types (modified from Spangenberg and Booth, 2003\(^3\))

<table>
<thead>
<tr>
<th>Classification scheme</th>
<th>Muscle fiber types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin heavy chain</td>
<td>Type I</td>
</tr>
<tr>
<td>Contractile speed</td>
<td>Type Ila</td>
</tr>
<tr>
<td>Energy metabolism</td>
<td>Type IIX</td>
</tr>
<tr>
<td>Muscle fiber color</td>
<td>Type IIB</td>
</tr>
<tr>
<td>Function</td>
<td>Postural</td>
</tr>
<tr>
<td>CSA</td>
<td>movement</td>
</tr>
<tr>
<td></td>
<td>movement</td>
</tr>
</tbody>
</table>

CSA = cross-sectional area

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**Figure 1.** Percentage of muscle fiber types in different muscle areas of the pig.\(^6,10,11\)

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growth rate of muscle fibers followed the trend that heavy pigs had higher total number of fiber (TNF) than middle and lightest weight pigs, which did not differ from each other.

**Breed**

Breed covers probably the main proportion of genetic factors affecting the muscle fiber composition of a certain muscle. The composition of muscle fiber types in different breeds is listed in table 2. Generally, the proportion of type IIb fibers is dominant in most modern and traditional breeds. However, a dramatic change in the relative expression profile was found in our study. Mongcai is a Vietnamese local breed and known for its highly preferred meat quality but unsatisfactory muscularity. Larger loin eye muscle area offered higher proportion of type IIb fibers and this helps to explain the difference of fiber distribution in Mongcai compared to other breeds. Moreover, in comparison between the numbers and types of muscle fibers in large and small pig breeds, authors announced that the responsible reasons for muscle size variation between LW and miniature pigs were due to the difference in myofiber number, a factor fixed before postnatal growth. In addition, it appeared that pigs exhibiting postnatal increases in myofiber size are more related to age than to live weight. The mechanism to which fewer muscle development in genetically small animals is different from that exhibited by nutritional deprivation animals in utero and consequences of these differences are therefore reflected in chemical properties of the constituent muscle fibers. In comparison between miniature and LW pigs, a higher content of type I fibers in the latter breed, which may support their increased weight and accord with the hypothesis that at similar stage of growth, there are great differences across breeds and fiber sizes. Finally, muscle of wild pigs was reported to contain higher area percentage of type IIa and conversely lower percentage area of type IIb fibers than those from the same muscle of domestic pigs.

**Gender**

There have been contradictory documentations on the distinction between females and males in term of proportion of muscle fibers. In general, females have larger fibers than castrated males and as a result, sex has been mentioned to affect on the cross-sectional area but not on the proportion of each fiber. Moreover, Lefaucheur et al demonstrated a significant decrease of relative fiber area of type I fiber in females compared to intact LW males, which may indicate that castration decreases relative area of type I fibers. Nevertheless, Rehfeldt et al evidenced smaller values of type IIa and IIb fibers in boars in comparison to female pigs and this implies a higher numbers of muscle fiber in male pigs because the weight of the longissimus dorsi muscle was similar.

**Birth weight**

The effect of birth weight on muscle fiber performances has been mentioned in many publications. Indeed, a tendency that lower total fiber number in piglets is associated with low birth weight was revealed. Most of the variations were due to a difference in the number of secondary myofibers that formed around each primary. However, results from
other studies did not observe any association between birth weight and TNF and thereby rejected this suggestion.\(^{27,28}\) Although birth weight was indicated to have an association with enlarged muscle fiber area,\(^{29}\) its influence on muscle fiber composition of longissimus dorsi and rhomboideus muscle was not established.\(^{10,22}\) Similarly, Rehfeldt \textit{et al}(2006)\(^{30}\)

Table 2. Breed-specific distribution of muscle fiber types

<table>
<thead>
<tr>
<th>Pig breeds</th>
<th>Muscle fiber type (%)</th>
<th>Method(^1)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>l</td>
<td>IIa</td>
<td>IIx</td>
</tr>
<tr>
<td>Modern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large White</td>
<td>6.2</td>
<td>7.5</td>
<td>23.4</td>
</tr>
<tr>
<td>Duroc</td>
<td>10.0</td>
<td>12.2</td>
<td>18.1</td>
</tr>
<tr>
<td>Pietrain</td>
<td>9.4</td>
<td>4.3</td>
<td>20.9</td>
</tr>
<tr>
<td>Landrace</td>
<td>13.2</td>
<td>9.0</td>
<td>-----77.9------</td>
</tr>
<tr>
<td>Yorkshire</td>
<td>9.9</td>
<td>8.6</td>
<td>-----81.5------</td>
</tr>
<tr>
<td>Traditional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berkshire</td>
<td>10.2</td>
<td>3.6</td>
<td>30</td>
</tr>
<tr>
<td>Tamworth</td>
<td>12.9</td>
<td>6.1</td>
<td>31.5</td>
</tr>
<tr>
<td>Meishan</td>
<td>8.3</td>
<td>13.4</td>
<td>61.1</td>
</tr>
<tr>
<td>Mongcai</td>
<td>24.1</td>
<td>28.6</td>
<td>35.9</td>
</tr>
<tr>
<td>Hampshire</td>
<td>15.2</td>
<td>9.4</td>
<td>-----75.3------</td>
</tr>
<tr>
<td>Wild boar</td>
<td>7.0</td>
<td>8.8</td>
<td>-----84.2------</td>
</tr>
<tr>
<td>Crossbred</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DUMI</td>
<td>25.9</td>
<td>7.4</td>
<td>12.7</td>
</tr>
<tr>
<td>DUPI</td>
<td>15.0</td>
<td>8.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Pi x LW</td>
<td>13</td>
<td>10</td>
<td>-----75.0------</td>
</tr>
<tr>
<td>Du x (Y x LW)</td>
<td>7.9</td>
<td>11.9</td>
<td>-----80.2------</td>
</tr>
</tbody>
</table>

\(^1\) Four fiber types can be detected by either Real-time PCR or \textit{in situ} hybridization (ISH) while only three isoforms are distinguished by mATPase. (Du = Duroc, DUMI = Duroc x Berlin Miniature Pigs, DUPI = Duroc x Pietrain, Pi = Pietrain, LW = Large White, Y = Yorkshire)
stated that the ranking of fiber number at slaughter was almost the same as at birth, with low fiber numbers in low birth weight and high numbers in high birth weight piglets. Because no differences were observed in the frequencies of different fiber types, Rehfeldt and Kuhn (2006) concluded that postnatal fiber differentiation is independent of birth weight.

**Age and slaughter weight**

At birth, muscle fibers are oxidative and the relative number of slow-twitch numbers continues to increase until 8 weeks after. The proportions of white fibers were observed to intensively increase up to 4 months of age, together with the rapidly increased size and continue at a slower rate afterward. Surprisingly, although type IIa fibers are a minor in porcine skeletal muscle but their relative fiber type-restricted expression was highest among 4 isoforms at both stages 6 weeks and 22 weeks postnatal. A relation of age and muscle fibers was additionally recorded in such a way that increasing weight and age at slaughter did change the cross-sectional area but not the numerical percentage of any fibers.

**Candidate genes**

Muscle fiber formation occurs during embryonic development including two stages of primary and secondary generation. During this period, the meiogenesis is under control of the MyoD gene family consisting of four structural related genes MYOD1 (MYF3), myogenin (MYOG or MYF4), MYF5 and MRF4 (MYF6). The MYOD1 and MYF5 genes are involved in myoblasts proliferation and they were found to directly affect the proportion of fast-twitch oxidative fibers and the fast-twitch low-oxidative fibers of pigs being crosses of Pietrain x (Polish LW x Polish Landrace) and thereby also influence the metabolic properties of muscle. The other gene, myogenin, is expressed in all myoblasts starts from differentiation to cell fusion and also marks the end of the myoblast proliferation. In deed, a significant difference of two homozygous genotypes for birth weight, growth rate and lean weight was reported by te Pas et al. (1999). The expression of the last gene in the MyoD family, MYF6, also involves in the differentiation and maturation of myotubes and highly expressed postnatally but experimental outcome demonstrating its effects on muscle fibers are still scare.

It is well known that pigs homozygous for the halothane (HAL) sensitivity allele (nn) are highly stress susceptible and often induces accelerated pH-ultimate (pHₜ₈) decline post-mortem (p.m.) leading to a pale, soft and exudative (PSE) meat. Different halothane genotypes have been described to associate with the content of muscle fibers and thereby affect on meat quality. For instance, Depreux et al. (2002) showed a greater amounts of type IIb but less slow fibers in pigs carrying the “n” gene (Nn or nn), whereas the NN pigs exhibited higher proportion of type IIax fibers. Moreover, a positive correlation between the relative abundance of type IIb fibers and pH was observed in pigs free of “n” gene, but across all genotypes, the relationship between type IIb fibers and pHₜ₈ was negative. This was curious because, it is opposite with the whole assumption that type IIb isoform hydrolyzes ATP rapidly and increases the glycolysis rate. In the other hand, it was suggested that the rela-
tive amounts of individual fibers are not attributed by the effects of HAL, or in other words, the process of maturation from one fiber to another is free from the HAL accelerated effect.\(^{(42)}\)

Conversely but similar detrimental effect on meat quality, the RN gene mainly increase the CSA of red fibers leading to a decrease in relative area of white muscle (IIX and IIB) and thus the fibers are more to oxidative and less to glycolytic metabolism. The RN effect, namely, “acid meat” phenotype indicated a positive correlation between glycolytic potential and lactate content and pH\(_{\text{u}}\).\(^{(43)}\) In fact, results from Marinova \textit{et al.}(1992)\(^{(44)}\) proved that pigs carrying RN gene have higher glycogen content in white muscles especially in glycolytic fibers. This was later confirmed by Lebret \textit{et al.}(1999),\(^{(45)}\) who concluded that white muscles are more affected than red muscles (I and IIA) and that the glycogen content increase in \textit{longissimus} muscle. Further findings from these authors also emphasized a higher enzyme activities and relative area of type II red fibers in the dominant RN carriers. A causative mutation (R200Q) for the RN gene in the \textit{PRKAG3} gene encoding for a muscle-specific isoform of the regulatory\(^{(33)}\) subunit of adenosine monophosphat-activated protein kinase additionally reported.\(^{(46)}\)

Calpastatin (CAST) is a specific inhibitor of calpain, a Ca\(^{2+}\)-activated protease family, considered to be involved in the initiation of myofibrillar protein degradation in living muscle.\(^{(47)}\) In a preliminary study, Klosowska \textit{et al.}(2005)\(^{(48)}\) showed that \textit{longissimus lumborum} diameters of all types of muscle fibers are significantly related to the Stamboek pigs’ genotype at locus CAST. The percentage of fast-twitch glycolytic fibers in a bundle was also concluded to change the metabolic properties of muscles and thereby meat quality. Although this locus is located in the 6\(^{th}\) intron of the gene, this intronic mutation should be considered both as a marker for muscle microstructure characteristics and as the causal mutation itself.\(^{(48)}\)

**Environmental factors**

**Prenatal feeding**

During gestation, prenatal muscle development includes two successive generations and thus produces primary fibers (up to 50-55 days) and secondary fibers (up to 90 days). In most cases, the nutritional manipulation has little effect on the early period of myogenesis, a stage involves in differentiation of primary muscle fibers. However, nutrition may change the number of primary fiber differentiation possibly because of indirect influence on the placenta development.\(^{(50,49)}\) In pigs, between 25 to 90 days of gestation, the differentiation and hyperplasia of secondary fibers have been demonstrated as a cause for under-nutrition, which can lead to runting, a decrease in muscle fiber numbers, especially secondary fibers.\(^{(25,27,50)}\) Likewise, findings on the relationship between over-nutrition and muscle fiber characteristics are still unclear. Over-nutrition of the sow between 25 to 50 days of gestation might increase the TNF in developing pigs\(^{(51)}\) whereas increased maternal nutrition of sows from day 25 to 50, or 25 to 70 of gestation did not give any benefits on muscle fiber number and area in the offspring.\(^{(52)}\)

**Postnatal feeding**

There has been much attention on the role of nutrition on muscle development. Under-
nutrition was demonstrated to account for a reduction of cross-sectional area of future fast-twitch glycolytic fibers in *longissimus dorsi* muscle.\(^{(53)}\) Chilibeck *et al* (2005)\(^{(54)}\) carried out an experiment on rapidly growing gilts, in which limited overfeeding at 75% more energy than needed for weight maintenance were offered at two stages, from days 1-7 (early luteal phase) and from day 8-15 (late luteal phase) of the estrous cycle. Results showed that muscle fiber area and fiber type composition were independent on restricted overfeeding at any time exclusive of a significant decrease in type IIa fiber percentage over time. Partly opposite results were presented by Chilibeck *et al* (2005),\(^{(55)}\) who failed to evaluate any effect of restricted feeding (approximately 30%, started from 30 to 100 kg) on fiber number, CSA diameter and relative area of fast-twitch oxidative, fast-twitch glycolytic fibers. However, a significant difference was noted in case of slow-twitch oxidative fibers, in which restricted pigs had larger cross-sectional area, diameter and relative area than those from *ad libitum* fed pigs. Supportably, an association between postnatal nutritional status and type I fiber expression both at mRNA and protein levels was unraveled.\(^{(56)}\) Also, restricted feeding (50% of *ad libitum*) at an early stage (between 3 and 7 weeks of age) had no influence in myofiber type composition in *longissimus dorsi* but led to a remarked increase in type I fibers proportion in the red *rhomboideus* muscle.\(^{(57)}\) An assumption for these findings is, because the energy usage per unit tension is lower in type I fibers, as a result, a selective increase in type I proportion in muscle during the period of reduced available energy would be physiologically relevant to spare energy.\(^{(23,57)}\)

**Other factors**

Other factors that may have an impact on muscle fiber characteristics include physical activity, ambient temperature and growth-promoting agents. In fact, climate conditions and physical exercise can influence on pig performance raised in an outdoor production system. Animals born outdoor at low temperature had a higher percentage of type I, but lower percentage of type IIa fibers in the *longissimus* muscle. Interestingly, this difference changes in pigs finished outdoor environment as Harrison *et al.* (1996)\(^{(58)}\) indicated a higher proportion of type IIa fibers in both *longissimus* and *semimembranosus* muscle, and vice versa for type IIb/IIx fibers. An increase of type I fiber percentage in pigs long-term exposing to cold temperature is generally accompanied by an increase in oxidative metabolism.\(^{(59)}\) Moreover, outdoor pigs have more spontaneous activities leading to a shift of muscle fibers from type IIb to IIx to IIa and to I, respectively, which can explain the more type IIa and less type IIb/IIx in muscle compared with indoor pigs.\(^{(23)}\) In addition to some environmental factors, growth promoters such as growth hormone, b-agonists and steroids can influence muscle fiber composition of farm animals. An excellent review regarding to these promoting agents is available.\(^{(60)}\)

**Conclusion**

It is clear that the distribution of skeletal muscle fibers is heterogeneous among individuals. However, conclusions whether these differences are genetically determined or
consequence of environmental influences are still controversial. Among genetic factors, candidate genes play an important role and have been used both inclusively and extensively in industry. On the other hand, prenatal nutrition is preferred in altering the fiber composition or increasing the total number of fibers as this factor is fixed before birth and known to positively relate to postnatal muscle growth.

References


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