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Possible Muscle Fiber Characteristics in the Selection for Improvement in Porcine Lean Meat Production and Quality

J. M. Kim, Y. J. Lee, Y. M. Choi[1], B. C. Kim[1], B. H. Yoo[2] and K. C. Hong*
Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

ABSTRACT : The aim of this study was directed at exploring the possible use of muscle fiber characteristics as new selection traits for improving both porcine lean meat production and quality. A total of 174 (114 Yorkshire, 30 Landrace, and 30 Meishan) pigs were used for this study, and lean meat production ability was estimated by backfat thickness and loin eye area. The *Longissimus dorsi* muscle was taken in order to measure meat quality and muscle fiber characteristics. Due to the high correlations between total muscle fiber number and most of the performance traits, all pigs were classified into three groups (low, intermediate, or high) by total muscle fiber number using cluster analysis. The high group had the highest loin eye area ($p<0.001$). The meat quality traits were within normal ranges as reddish pink, firm, and nonexudative (RFN) pork, but the groups classified as intermediate and high had relatively large drip loss percentages ($p<0.05$), produced more than twice the amount of pale, soft, and exudative (PSE) pork as compared to the low group. The group with a high total muscle fiber number was further classified, based on type 2b fiber percentage, into low or high groups by cluster analysis. The results showed that the low type 2b fiber group had good loin eye area ($p<0.05$), small drip loss ($p<0.05$), and did not produce PSE pork. For these reasons, a high total muscle fiber number, with a low percentage of type 2b fibers, may be suitable in selecting for improvements in both lean meat production and meat quality. (**Key Words :** Muscle Fiber Characteristics, Lean Meat Production Ability, Meat Quality)

INTRODUCTION

The goals of pig breeding are directed not only toward producing lean meat for retail, but also toward meat quality. However, during the last few decades, breeding programs are focused on selecting for rapid lean meat production (Brocks et al., 2000), which is influenced by several interacting genes. In some breeds, accompanying results included decreased stress resistance and poor meat quality (Cameron, 1990; Jin et al., 2006). To counteract this unfavorable development, it has been advantageous to consider new selection traits that positively relate to both muscle growth and meat quality (Lengerken et al., 1994). Several possible new selection traits have been suggested based on functional muscle properties (Lengerken et al., 1994; Maltin et al., 1997; Morel et al., 2006). One faction of these is represented by muscle fiber characteristics, which are well-described with respect to their morphological and physiological properties, as well as their importance for meat quality (Fiedler et al., 1999).

Among the muscle fiber characteristics, the total number of muscle fibers is an important factor affecting muscle mass and meat quality (Rehfeldt et al., 2000). The number of muscle fibers is important in terms of the growth potential of skeletal muscle, as well as for endurance fitness and the ability to adapt to environmental stress in farm animals, and also important for meat content and meat quality development after slaughter (Rehfeldt et al., 1999). In several studies it was reported that most muscle fiber characteristics have medium heritability and significant genetic correlations with both lean meat production and meat quality traits (Larzul et al., 1997; Fiedler et al., 2004).

Additionally, type of muscle fiber can affect the meat quality by influencing the post mortem changes during conversion of muscle into meat (Karlsson et al., 1999; Brocks et al., 2000; Ryu and Kim, 2006). The functional, structural, and metabolic characteristics of the three major fiber types are different in adult animals. Red muscle fibers

* Corresponding Author: K. C. Hong. Tel: +82-2-3290-3053, Fax: +82-2-925-1970, E-mail: kchong@korea.ac.kr
[1] Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, 5-1 Anam-dong, Sungbuk-gu, Seoul 136-701, Korea.
[2] Sangwon Pig Development Company, 11 San, Tae-ri, Gochon-myeon, Gimpo-si, Gyeonggi-do 415-812, Korea.
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(type 1) are rich in myoglobin content and have a high oxidative and low glycolytic metabolic capacity, whereas intermediate (type 2a) and white (type 2b) fibers are fast twitching and have low oxidative and high glycolytic metabolic activity (Essen-Gustavsson et al., 1994).

The aim of this study was to examine the possibility of using muscle fiber characteristics as new selection traits for both improving lean meat production ability and meat quality. For this purpose, we analyzed the correlations between muscle fiber characteristics and the lean meat production and quality traits in pork. Based on the results, we further examined the relationships by classifying the total muscle fiber number with fiber type composition by cluster analyses.

MATERIALS AND METHODS

Animals

A total sample of pigs that consisted of 114 Yorkshire (79 females and 35 castrated males), 30 Landrace (19 females and 11 castrated males), and 30 Meishan (16 females and 14 castrated males) was evaluated in this study. The pigs were slaughtered during three age groups 172.6±5.1 d, 174.6±5.4 d, and 176.8±8.8 d, respectively. Backfat thickness was measured at the 11th and last *thoracic vertebrae*. The mean of these two measurements was used as the backfat thickness value. The loin eye area was measured at the level of the last rib.

Meat quality measurements

The carcasses were chilled at 4°C for 24 h and the *longissimus dorsi* muscle was taken to evaluate the meat quality traits. Drip loss was determined by suspending the muscle samples, standardized for surface area, in an inflated plastic bag for 48 h at 4°C (Honikel, 1987). The lightness (L*) of the meat was measured at the 8th-9th *thoracic vertebrae* at 24 h postmortem with a chromameter (CR-300, Minolta Camera Co., Japan). The pH value was measured at the location of 13th/14th rib using a spear-type electrode (Model 290A, Orion Research Inc., USA) at 45 min postmortem.

Analyses of muscle fiber characteristics

At 45 min postmortem, a sample was taken from the *longissimus* muscle at the 8th *thoracic vertebrae*. Samples were cut into 0.5×0.5×1.0 cm pieces, promptly frozen in isopentane cooled by liquid nitrogen, and stored at -80°C until subsequent analyses. Transverse serial sections (10 μm thick) were cut on a cryostat instrument (CM1850, Leica, Germany) at -20°C, and then stained for actomyosin ATPase after acid pre-incubation (pH 4.35) in order to identify the type 1, 2a, and 2b fibers (Brooke and Kaiser, 1970). All muscle fiber samples were examined by an image analysis system. The operational system consisted of an optical microscope equipped with a charge-coupled device color camera (IK-642K, Toshiba, Japan) and a standard workstation computer that controlled the image analysis system (Image-Pro Plus, Media Cybernetics, USA). Fiber density was calculated from the mean number of fibers per mm^2. For the calculation of the total fiber number, the fiber density was multiplied with the loin eye area (cm^2).

Statistical analysis

Basic statistics were performed using the statistical software package SAS 9.13 (SAS Institute Inc., 2001) to calculate simple profile data. The correlation coefficients between the muscle fiber characteristics and other carcass traits were evaluated using the CORR procedure of SAS. Cluster analyses were conducted in order to generate the total fiber number classifications (low, intermediate, or high) and type 2b fiber composition classifications (low or high), using the FASTCLUS procedure in the SAS software package.

After classification, the general linear model procedure was performed for the association between groups and traits, using the software. The model was: $y_{ijklm} = \mu+G_i+S_j+B_k+e_{ijkl}$, where y_{ijklm} is the observation; μ is the general mean; G_i is the fixed effect of the group i; S_j is the fixed effect of sex j; B_k is the fixed effect of breed k; and e_{ijkl} is the random error. When significant differences were detected, the mean values were separated by the probability difference (PDIFF) option. The results are presented as least squares means for each group, together with the standard errors of the least squares means. Due to a small percentage (≤20%) of type 1 and 2a fibers, and a large percentage (≥80%) of type 2b fibers, the total number of fibers was calculated using logarithmic transformation, while the muscle fiber compositions were estimated using angular transformation.

RESULTS AND DISCUSSION

Data profile

All the measured traits in this study are presented by a number of measurements per trait, means with standard deviations, and their overall ranges (Table 1). All the traits are divided under three major categories: muscle fiber characteristics, lean meat production ability, and meat quality. The muscle fiber characteristic traits consist of total fiber number, mean cross-sectional area (CSA), and fiber type composition number; these all had large variations between individual pigs. The mean value for total fiber number was 1,186 ($\times10^3$), and the mean cross-sectional area of the fibers was 4,176 μm^2. The majority of fibers were type 2b at 79.40%. However, type 1 only made up 8.83% of the total fibers. According to the muscle fiber type compositions of the representing results, as would be

Table 1. Means and standard errors (SE) for muscle fiber characteristics, lean meat production ability, and meat quality

Traits	n	Mean	SE	Min	Max
Muscle fiber characteristics					
Total fiber number ($\times 10^3$)	174	1,186	23.04	644	2,413
Cross-sectional area of fibers	174	4,176	52.43	2,644	6,610
Fiber type composition number (%)					
Type 1 fiber	174	8.83	0.28	3.03	22.54
Type 2a fiber	174	11.79	0.34	3.34	25.63
Type 2b fiber	174	79.40	0.43	61.11	92.12
Lean meat production ability					
Backfat thickness (mm)	174	18.03	0.40	6.00	30.00
Loin eye area (cm^2)	174	48.20	0.67	31.11	77.60
Meat quality					
Muscle pH_{45min}	174	5.97	0.02	5.34	6.65
Drip loss (%)	174	4.51	0.20	0.52	13.31
Lightness (L^*)	174	47.50	0.20	41.74	57.73
PSE[1] (%)	18	(10.34)[2]			

[1] PSE (pale, soft, and exudative): lightness >50, drip loss >6.0%.
[2] Percentage of PSE pork in total pigs.

expected, the porcine *longissimus dorsi* muscle was determined to be a fast-twitch muscle. These results are analogous to previous studies (Larzul et al., 1997; Fiedler et al., 2004), although some of the units of value are different from each other.

Lean meat production ability was indicated by backfat thickness and loin eye area. The mean values were 18.03 mm and 48.20 cm^2, respectively. The measured traits for meat quality were drip loss (4.51%), ultimate muscle pH at 45 min postmortem (5.97), and lightness at 24 h postmortem (47.50). Although the mean values of the meat quality traits were in normal ranges as reddish pink, firm, and nonexudative pork (RFN; drip loss 2.0-6.0%, lightness (L*) 42-50), about ten percent (n = 18) of the total pigs produced pale, soft, exudative pork (PSE; drip loss >6.0%, lightness (L*) >50) (Joo et al., 1999).

Correlations of muscle fiber characteristics with lean meat production and meat quality traits

The correlation coefficients between the muscle fiber characteristics and the other traits are presented in Table 2. The total muscle fiber number (TN) typically correlated to the other traits. TN showed a negative relationship with the mean cross-sectional area (CSA; r = -0.65, p<0.05), which is in agreement with previous studies (Larzul et al., 1997). An opposite correlation between the percentages of type 1 fiber (TI) and type 2b fiber (TIIb) was observed with TN (r = -0.21 and 0.17, p<0.05). TN was calculated as the loin eye area multiplied by the fiber density, and these two parameters are affected by the simultaneous effect of carcass weight or muscle mass (Ryu et al., 2004). Our results also show that TN was positive and highly and significantly correlated with backfat thickness (BF; r = 0.45) and loin eye area (LE; r = 0.77). For the meat quality traits, TN was negatively correlated with muscle pH 45 min after slaughter (PH; r = -0.24), and positively correlated with drip loss (DL; r = 0.24).

The correlations between CSA and fiber type compositions showed opposite results with TN. CSA was positively correlated to TI (r = 0.28) and negatively correlated to TIIb (r = -0.23). The TI and TIIa fiber type compositions were negatively correlated to TIIb fibers (r =

Table 2. Simple correlations of muscle fiber characteristics with lean meat production ability and meat quality traits

Traits	Muscle fiber characteristics				
	TN	CSA	TI	TIIa	TIIb
CSA	-0.65*				
TI	-0.21*	0.28*			
TIIa	-0.04	0.06	-0.07		
TIIb	0.17*	-0.23*	-0.60*	-0.75*	
BF	0.45*	-0.10	-0.06	0.00	0.05
LE	0.77*	-0.06	-0.05	0.01	0.04
PH	-0.24*	0.09	0.10	-0.10	0.01
DL	0.24*	0.00	-0.13*	-0.11	0.18 *
L	0.03	0.11	-0.02	-0.11	0.12

Levels of significance at * p<0.05.
TN = Total fiber number; CSA = Cross-sectional area of fibers; TI = Type 1 fiber percentage; TIIa = Type 2a fiber percentage.
TIIb = Type 2b fiber percentage; BF = Backfat thickness; LE = Loin eye area; PH = Muscle pH_{45min}; DL = Drip loss; L = Lightness.

Table 3. Least square means and their standard errors of the total fiber number classes by cluster analysis on performance traits

Trait	Total fiber number class		
	Low (n = 95)	Intermediate (n = 56)	High (n = 23)
Total fiber number (×10^3)	1,038±22.3 [C]	1,368±21.5 [B]	1,798±29.5 [A]
Muscle fiber characteristics			
CSA[1] of fibers (μm^2)	4,556±101.6 [A]	3,939±98.1 [B]	3,403±134.6 [C]
Fiber type composition number (%)			
Type 1 fiber	11.49±0.61 [A]	9.76±0.59 [B]	7.58±0.81 [C]
Type 2a fiber	14.34±0.73 [b]	12.27±0.71 [a]	12.86±0.97 [ab]
Type 2b fiber	74.23±0.87 [A]	78.03±0.84 [B]	79.51±1.15 [B]
Lean meat production ability			
Backfat thickness (mm)	19.91±0.86	20.91±0.83	20.01±1.14
Loin eye area (cm^2)	46.98±1.01 [C]	54.34±0.97 [B]	61.46±1.33 [A]
Meat quality			
pH_{45min}	5.85±0.05	5.89±0.05	5.83±0.06
Drip loss (%)	3.92±0.42 [a]	5.15±0.41 [b]	5.24±0.56 [ab]
Lightness (L*)	47.33±0.43	47.54±0.42	46.88±0.57
PSE (%)[2]	6/95 (6.32) [3]	9 56 (16.07)	3/23 (13.04)

Values with different superscripts indicate significance levels within rows: [A, B] p<0.001, [a, b] p<0.05.
[1] Cross-sectional area. [2] PSE (pale, soft, and exudative): lightness >50, drip loss >6.0%.
[3] Number of PSE in a group and their proportion (%).

-0.60 and -0.75, respectively), which is in accordance with a previous study (Ryu et al., 2004). Because the porcine *longsissimus dorsi* muscle mostly consisted of type 2b fibers, there were relatively high correlations between TIIb and the other muscle fiber characteristics. However, with the exception of DL (r = 0.18), TIIb was not significantly related to the lean meat production ability or meat quality traits.

In previous studies, the genetic potential of muscle fiber characteristics was analyzed based on heritability and the correlations among traits (Larzul et al., 1997; Fiedler et al., 2004). The phenotypic correlations between the muscle fiber characteristics and other performance traits were also analyzed (Karlsson et al., 1993; Fiedler et al., 2003; Ryu and Kim, 2005). Most of the previous results showed that the muscle fiber characteristics had medium to high heritabilities, and were significantly genetic or phenotypic correlated with lean meat production and meat quality traits. Among the muscle fiber characteristics, the total number of fibers was considered as a new selection criterion for both improved muscle growth and meat quality (Rehfeldt et al., 2000; Fiedler et al., 2004). Our results also indicate that TN was significantly related to both the lean meat production ability and meat quality traits. Thus, we expect that the TN can affect both traits, which is in agreement with previous studies (Larzul et al., 1997; Rehfeldt et al., 2000; Fiedler et al., 2004).

Effects of total fiber number

All the assessed pigs were classified into three different groups by total fiber number using cluster analysis (R^2 = 0.89); then associations analysis was performed on the groups with the other performance traits (Table 3). As expected, the total numbers of fibers were highly and significantly different among these groups (p<0.001). The high group had the lowest CSA size for fibers (p<0.001), and percentage of type 1 fiber (p<0.001), whereas the low group had the lowest percentage of type 2b fiber among the groups (p<0.001). Backfat thickness was not significantly different among the groups; however, loin eye area was significantly different (p<0.001), with the highest mean value occurring in the high group.

For meat quality, although the traits were in the normal range as RFN pork, drip loss was significantly different between the groups (p<0.05). When compared to the low group, the intermediate group showed a relatively high percentage of drip loss, followed by the high group (but it was not significantly different). Moreover, the percentage of PSE pork produced by the intermediate and high groups was more than twice that of the low group. We believe these results relate to the significant correlation between total fiber number and drip loss.

In a previous study, a critical range for total fiber number, which guarantees both high lean meat production and good meat quality, was suggested for Piétrain pigs (Lengerken et al., 1997). The results showed that *longissimus* muscles with the highest number of fibers (>1,200-1,600, ×10^3) had good meat quality without a significant difference in lean meat production ability, including the lean meat percentage and loin muscle area. Our results are in agreement with these results by showing that the group with the highest total number of fibers had good lean meat production ability. However, it did not show a better meat quality than lower fiber number groups.

Table 4. Least square means and standard errors of the type 2b fiber percentage class, within the high total fiber number group, by cluster analysis on performance traits

Trait	Class based on percentage of type 2b fiber	
	Low (n = 8)	High (n = 15)
Fiber type composition number (%)		
Type 1 fiber	10.24±1.23*	4.90±1.42*
Type 2a fiber	13.44±1.88	12.56±2.16
Type 2b fiber	76.32±2.20*	82.54±2.53*
Muscle fiber characteristics		
Total fiber number (×10^3)	2,038±98.2**	1,620±112.8**
CSA[1] of fibers (μm^2)	3,417±251.4	3,469±288.9
Lean meat production ability		
Backfat thickness (mm)	18.97±2.64	18.83±3.04
Loin eye area (cm^2)	69.08±3.20*	56.04±3.67*
Meat quality		
pH_{45min}	5.75±0.14	5.76±0.17
Drip loss (%)	3.67±0.82*	5.92±0.59*
Lightness (L*)	46.94±1.72	47.90±1.98
PSE (%)[2]	0/8 (0.00)[3]	3/15 (20.00)

Values with different superscripts indicate significance levels within rows: * p<0.05, ** p<0.1.
[1] Cross-sectional area. [2] PSE (pale, soft, and exudative): lightness >50, drip loss >6.0%.
[3] Number of PSE in a group and their proportion (%).

Effects of type 2b fiber percentage within the high total muscle fiber group

Meat quality has large individual variation both within and between pigs of the same breed, sex, and age (Lawrie, 1998; Kim et al., 2004). Higher percentages of fast-twitch fiber are shown to correlate with PSE (pale, soft, exudative) pork, meat condition (Linke, 1972; Larzul et al., 1997; Fiedler et al., 1999), and with stress susceptibility (Nelson and Schochet, 1982; Fiedler, 1993; Ryu and Kim, 2006). The porcine *longissimus dorsi* muscle is a fast-twitch muscle; therefore, we considered that the type 2b fiber percentage may directly affect meat quality.

The group with the highest total fiber number was further classified into two groups based on type 2b fiber percentage by cluster analysis (R^2 = 0.77, Table 4). In fiber type composition number, their type 1 and 2b fiber percentages were significantly different, with the low group having more type 1 fiber ($p<0.05$) and less type 2b fiber than the high group ($p<0.05$). Also, the low group had a higher total number of fibers than the high group ($p<0.1$). From the perspective of lean meat production ability, although backfat thickness was not significantly different, the low group showed better results than the high group, where the loin eye area of the low group was larger than that of the high group ($p<0.05$). Simultaneously, the low group showed a relatively small percentage of drip loss ($p<0.05$) for the meat quality traits. Moreover, the low group did not produce any PSE pork. Therefore, the data indicate that a lower percentage of type 2b fibers (low group), within the highest total fiber number group, resulted in good lean meat production ability as well as good meat quality.

For using muscle fiber characteristics as new selection traits, previous studies have presented results on the genetic and phenotypic correlations among these characteristics, or between other lean growth and meat quality traits (Larzul et al., 1997; Fiedler et al., 2004; Ryu et al., 2004; Ryu and Kim, 2005). In this study, additional attention was directed toward exploring the possibility of using the total number of fibers as a new selection trait for improving both lean meat production and meat quality. Proceeding from what was presented above we can conclude that the total fiber number and type 2b fiber percentage are practical new selection traits. Moreover, we suggest that a high total muscle fiber number along with a low percentage of type 2b fibers is a suitable selection criterion. Additionally, further studies on selection should point toward the development of new DNA molecular markers based on this range of traits within a living body.

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