Estimation of pork quality in live pigs using biopsied muscle fibre number composition

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A B S T R A C T

Here, we newly provided the parameters for estimating meat quality in live pigs using the muscle biopsy. The biopsied *longissimus thoracis* muscle was used to identify the muscle fibre characteristics (MFCs). Of the various MFCs in biopsied muscle, muscle fibre number (MFN) composition showed the greatest correlation with the MFCs in postmortem muscle ($P < 0.001$). Moreover, the pigs cluster groups, based on the biopsied MFN composition, demonstrated statistically significant differences in meat quality traits such as muscle pH, drip loss, and meat colour ($P < 0.05$). Therefore, we conclude that the MFN parameters in live pigs are closely related to the postmortem muscle metabolic rate and ultimately with the quality of meat. We suggest that the higher type I and lower type IIB MFN in biopsied muscle will result in better pork quality.

1. Introduction

Recently, pork has become the highest percentage of consumed meat worldwide (USDA annual reports, 2014), and meat quality is still the most important factor for customer satisfaction (Xiong, Sun, Pu, Gao, & Dai, 2017). Meat quality, however, has conventionally been evaluated postmortem, and as a result, there are limitations in pig selection and/or sustenance of brand uniformity. The estimation of meat quality in live pigs would, therefore, have important implications in both breeding and commercial farms. Although intramuscular fat (IMF) content is can be measured in live pigs by ultrasonic waves (Ayuso, Gonzalez, Hernandez, Corral, & Izquierdo, 2013), the major characteristics for determining pale, soft and exudative (PSE) pork, such as muscle pH, water holding capacity, and lightness are still not measurable in live animals.

Muscle consists of muscle fibres, which are classified as type I, type IIA, and type IIB according to their metabolic properties (Brooke & Kaiser, 1970; Lefaucheur, 2010). Numerous studies have revealed that the muscle fibre type composition in the *longissimus thoracis* (LT) loin muscle is closely associated with meat quality (Henckel, Oksbjerg, Erlandsen, Barton-Gade, & Bejerholm, 1997; Ryu & Kim, 2005; Warner, Kauffman, & Russel, 1993). A large proportion of type I fibres, especially, can favorably affect the muscle to meat conversion, because type I fibres have slow, aerobic metabolisms which delay the postmortem metabolic rate (Lefaucheur, 2010).

Biopsy has long been used in medical diagnostics and muscle biopsy, in particular, has been utilized in numerous studies evaluating exercise response and aging in humans (Frontera et al., 2000; Louis, Raue, Yang, Jemiolo, & Trappe, 2007). In pigs, the muscle biopsy method has been previously described for IMF analysis in pork loin (Ville et al., 1992). Moreover, muscle biopsy has been used to classify red and white fibre types in lamb (Valin, Touraille, Vigneron, & Ashmore, 1982).

In this study, we have biopsied the LT muscle in live pigs and examined the muscle fibre characteristics (MFCs). We then compared the biopsied MFCs with both the postmortem MFCs and the final meat quality traits in the slaughtered LT muscle. The purpose of this study is to define parameters using the biopsied MFC values of live pigs to estimate ultimately the postmortem meat quality. We believe this study will lay the ground work for further studies looking at ways to estimate potential meat quality in live animals.

2. Materials and methods

2.1. Animals and muscle samples

All experimental procedures were approved by the Committee on the Ethics of Animal Experiments of Jeju National University. A total of 613 Jeju black commercial pigs (152 castrated males, 80 intact males, and 381 females) were used in this study. Pigs were fed with the same
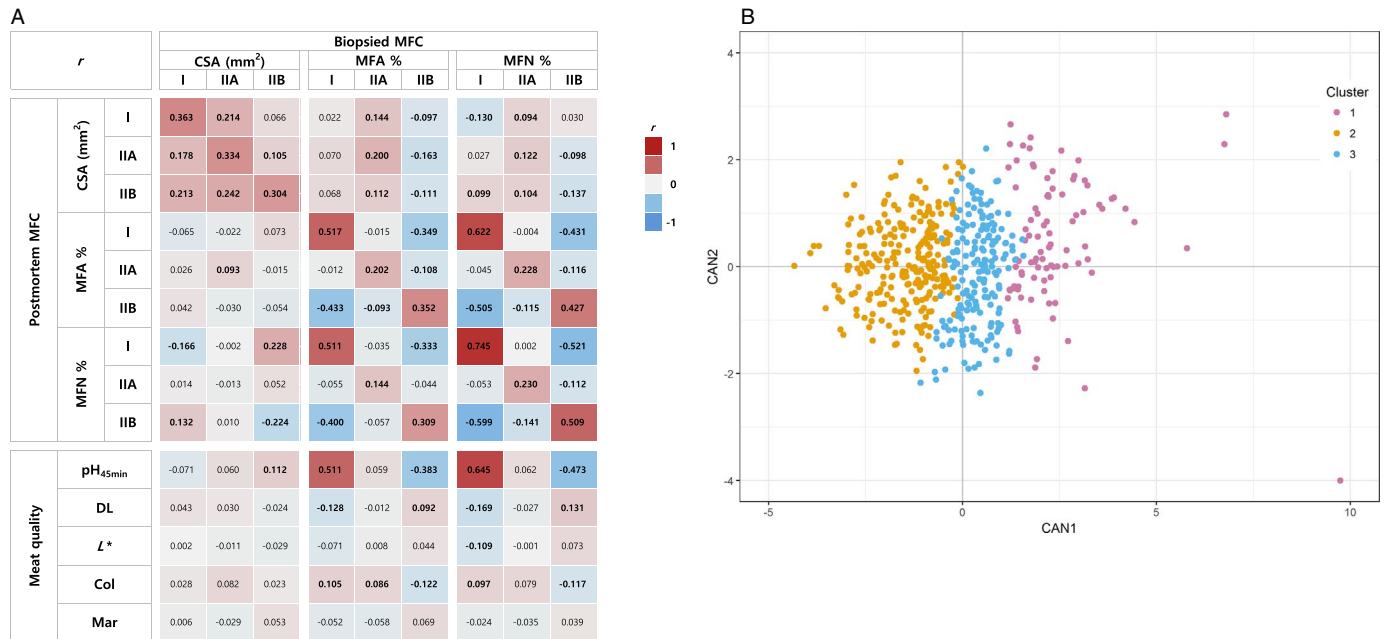

Fig. 1. Characterization of MFN composition in biopsy. (a) A heatmap for correlations of biopsied MFN with postmortem MFC and meat quality. The colour scale bar from red to blue represented the levels of correlation coefficients. Significant correlation coefficients ($P < 0.05$) were marked in bold. (b) A scatter plot by two dimensional canonical coefficients for three clusters (1, blue; 2, green; 3, red). Pigs from the clusters were classified by proportion of MFN variables. MFN, muscle fibre number; MFC, muscle fibre characteristics; CSA, cross-sectional area; MFA, muscle fibre area; DL, drip loss, Col, NPPC colour; Mar, NPPC marbling. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

feeding conditions in different pens of the same farm. When the pigs were moved to the fattening pen at 106.9 ± 7.9 days, we took a biopsy of the *longissimus thoracis* (LT) muscle upon around the 8th to 9th thoracic vertebrae by a disposable biopsy needle with a minimal length of 4 cm and a diameter of 1.8 mm (Guideneedle, Truguide, Bard, USA) under local anesthesia and aseptic conditions. Then, the pigs were slaughtered at 213.5 ± 17.5 days according to a standard protocol. At 45 min postmortem, the LT muscle samples from the 8th to 9th thoracic vertebrae were sampled and promptly frozen with liquid nitrogen to measure MFCs. After 24 h of chilling at 4 °C, the LT muscle from the 10th to 13th thoracic vertebrae was sampled for other meat quality traits measurement.

2.2. Measurement of muscle fibre characteristics in biopsy and postmortem

For the biopsied and postmortem LT muscle samples, the fibre types were classified in this study according to myofibrillar adenosine triphosphatase staining methods (Brooke & Kaiser, 1970). Next, an optical image analysis program (Image-Pro Plus, Media Cybernetics, L.P., USA) was employed to analyze the histochemical images. The cross-sectional area (CSA) of the muscle fibre was expressed as the ratio of the total area of measured muscle fibres to the total fibre count.

2.3. Measurement of meat quality

Muscle pH at 45 min postmortem was measured by a spear-type portable pH meter (HM-17MX, Toadkk, Japan) which was calibrated by standard solutions of pH 4 and pH 7 with automatic temperature compensation. The LT muscles were then chilled at 4 °C for 24 h and later used to evaluate other meat quality traits. To calculate drip loss, the fresh meat samples (about 80–100 g) were cut and immediately weighed (initial weights). The samples were suspended in an inflated bag at 4 °C for 48 h then weighed after being gently blotted dry (final weight). Drip loss measurements were expressed as a percentage of the initial sample weight. For colour and marbling measurements, muscle samples were bloomed by exposing them to air at 4 °C for 30 min. After

triplicate measurements using a Minolta chromameter (CR-300, Minolta Camera Co., Japan), the average values were expressed in Commission Internationale de l'Eclairage (*C.I.E.*, 1978) lightness units (*L**). CR-300 has 8 mm aperture with open cone, and the illuminant used was C. The standard observer position was 2°. The meat colour (1 to 6 correspond with pale to dark) and marbling score (1 to 10 correspond with low to high) for fresh pork according to National Pork Producers Council (NPPC) standards (2000) were assessed by 12 panelists on samples exposed to air at 4 °C for 30 min.

2.4. Statistical analysis

Statistical software package SAS 9.2 (SAS Institute Inc.) was used to calculate the correlation coefficients and analyze associations. The pigs were clustered based on three variables of muscle fibre number (MFN) composition in a two-step process: First, the FASTCLUS procedure in SAS was used to make ten pre-clusters, then the CLUSTER procedure was used to classify the pre-clusters hierarchically (Fig. S1) by the Ward's minimum-variance method (Ward, 1963). Finally, three clustered pig groups were used to estimate the association between the biopsied MFN composition and the postmortem MFCs and meat quality traits. The general linear model used to analyze the association between the clusters and the measured traits was: $y_{ijklm} = \mu + C_i + S_j + D_k + P_l + e_{ijklm}$, where y_{ijklm} is the observation of the traits, μ is the general mean, C_i is the fixed effect of cluster i, S_j is the fixed effect of sex j, D_k is a covariation for sampling age in biopsy or slaughter k, P_l is the fixed effect of sire and e_{ijklm} is the random error. The results were presented as least-square means and standard errors and significant differences between the cluster groups were tested by the probability difference (PDIFF) option.

3. Results and discussion

3.1. Biopsied MFCs and postmortem MFCs

A total of 613 pigs were used in this study, and muscle sampling for