TITLE PAGE - Food Science of Animal Resources -Upload this completed form to website with submission

| ARTICLE INFORMATION | Fill in information in each box below |
|--|--|
| Article Title | Muscle fiber characteristics on chop surface of pork loin (M. longissimus thoracis et lumborum) associated with muscle fiber pennation angle and their relationships with pork loin quality |
| Running Title (within 10 words) | Muscle fiber architecture and pork loin quality |
| Author | Sumin Song1, Huilin Cheng1, Eun-Young Jung2, Seon-Tea Joo3, Gap-Don Kim1,2 |
| Affiliation | Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea Institutes of Green Bio Science & Technology, Seoul National University, Pyeongchang 25354, Republic of Korea Division of Applied Life Science (BK21+), Gyeongsang National University, Jinju 52852, Republic of Korea |
| Special remarks – if authors have additional information to inform the editorial office | |
| ORCID (All authors must have ORCID) https://orcid.org | Sumin Song (https://orcid.org/0000-0001-7115-2253) Huilin Cheng (https://orcid.org/0000-0003-0628-3358) Eun-Young Jung (https://orcid.org/0000-0001-5739-4280) Seon-Tea Joo (https://orcid.org/0000-0002-5483-2828) Gap-Don Kim (https://orcid.org/0000-0001-5870-8990) |
| Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.) | The authors declare no potential conflict of interest. |
| Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.) | This work was supported by the National Research Foundation of Korea (NRF grant funded by Korea government (MSIT) (NRF-2019R1C1C1011056). |
| Author contributions (This field may be published.) | Conceptualization: Km GD, Song SM, Joo ST; Data curation: Kim GD, Song SM; Formal analysis: Song SM, Cheng H, Jung EY; Methodology: Song SM, Cheng H, Jung EY; Writing – original draft: Song SM; Writing – review & editing: Kim GD, Song SM, Cheng H, Jung EY, Joo ST. |
| Ethics approval (IRB/IACUC) (This field may be published.) | This manuscript does not require IRB/IACUC approval because there are no human and animal participants. |

L 5 6

CORRESPONDING AUTHOR CONTACT INFORMATION

| For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints) | Fill in information in each box below |
|--|--|
| First name, middle initial, last name | Gap-Don Kim |
| Email address – this is where your proofs will be sent | gapdonkim@snu.ac.kr |
| Secondary Email address | |
| Postal address | Graduate School of International Agricultural Technology, Seoul National University, 1447 Pyeongchangdae-ro, Pyeongchang 25354, Republic of Korea |
| Cell phone number | +82-10-3233-5840 |
| Office phone number | +82-33-339-5778 |
| Fax number | +82-33-339-5779 |

- Muscle fiber characteristics on chop surface of pork loin (M. *longissimus thoracis et lumborum*) associated with muscle fiber pennation angle and
 their relationships with pork loin quality
- 10

11 Abstract

The influence of muscle architecture on muscle fiber characteristics and meat quality 12 has not been fully elucidated. In the present study, muscle fiber characteristics on the chop 13 surface of pork loin (M. longissimus thoracis et lumborum, LTL), pennation angle degree, 14 15 and meat quality were evaluated to understand the pork LTL architecture and its relationship with the loin chop quality. Muscle fiber pennation degree ranged from 51.33° to 69.00°, 16 resulting in an ellipse-shaped muscle fiber on the surface of pork loin chop. The cross-17 sectional area (CSA) on the sections cut vertical to the muscle length (M-Vertical) was 18 considerably larger (p < 0.05) than that on the sections cut vertical to the muscle fiber 19 20 orientation (F-Vertical) regardless of the fiber type. Pennation angle is positively correlated with CSAs of F-Vertical (p < 0.05) and with Warner-Bratzler shear force (r = 0.53, p < 0.01). 21 Besides the shear force, lightness and pH were positively correlated with the fiber 22 23 composition and CSA of IIX fiber (p<0.05); however, the redness, yellowness, drip loss, and cooking loss were not correlated with the pennation angle and muscle fiber characteristics on 24 the chop surface (p>0.05). These observations might help us in better understanding pork loin 25 26 architecture and the relationship between the pennation angle, muscle fiber characteristics, and meat quality of pork loin chop. 27

28

29 Keywords: muscle architecture; pennation angle; muscle fiber characteristics; pork loin

30 **1. Introduction**

Muscle fiber type distribution, relative composition, fiber size, total number, and 31 32 fiber density have been considered as the major criteria for assessing meat quality, growth performance, and carcass characteristics of livestock animals (Bee et al., 2007; Chang et al., 33 2003; Choi and Kim, 2009; Hwang et al., 2010; Joo et al., 2013; Lebret et al., 1999; 34 Lefaucheur et al., 2002; Ozawa et al., 2000; Ryu and Kim, 2006). These muscle fiber 35 characteristics are assessed based on the muscle functions in the body, thereby resulting in 36 different physiological and morphological properties of each muscle (MacIntosh et al, 2005; 37 McDonald et al., 1997). Individual muscles have their own muscle fiber architecture and are 38 classified into several types, such as fusiform, pennate, and parallel muscles (Nigg and 39 40 Herzog, 1994; Narici, 1999). Among the skeletal muscles, present in the mammalian body, M. longissimus thoracis et lumborum (LTL) is located on the vertebrae and supports and 41 regulates the body movements. Accordingly, the LTL muscle is pennate type and its 42 pennation angle ranges from 31.36° to 83.33° regardless of the species (Derington et al., 43 2011; Kim et al., 2018). Owing to the diverse muscle fiber architecture, muscle fibers of 44 45 inconsistent faces are exposed to the muscle surface while cutting meat pieces or chops. In pork loin chops, an oval shape is predicted due to the leaned muscle fibers in the LTL muscle. 46 Moreover, a changeable shape is expected due to the angle degree of muscle fiber pennation 47 while considering the pennation angle ranges from 48.00° to 83.33° in porcine LTL muscle 48 (Kim et al., 2018). 49

Previous studies on muscle fiber characteristics have mainly focused on their
relationships with carcass traits and meat quality (Chang et., 2003; Choi and Kim, 2009;
Hwang et al., 2010; Joo et al., 2013; Kim et al., 2010; Kim et al., 2013a, 2013b; Ozawa et al.,
2000; Ryu and Kim, 2006); however, skeletal muscle morphology and muscle fiber

54 architecture have not been fully addressed from the meat science point of view. In addition, loin steak is normally prepared by cutting vertically to the muscle length. Consequently, meat 55 quality characteristics, such as color, water-holding capacity, and tenderness may be affected 56 by the chop surface condition, especially the characteristics of the muscle fibers on the chop 57 surface, as it has been demonstrated in beef striploin influenced by the muscle fiber angle 58 59 (Derington et al., 2011). Therefore, in the present study, the degree of pennation angle was evaluated to understand the morphological characteristics (muscle fiber architecture) of 60 61 porcine LTL muscle and its influence on the muscle fiber characteristics of the pork loin chop surface. Furthermore, their relationships with meat quality in pork loin chop was assessed. 62 63

- -
- 64

65 2. Materials and methods

66 2.1. Sample preparation

Pig carcasses (n = 20, crossbred of Landrace × Yorkshire × Duroc, 82.5 ± 3.8 kg of 67 carcass weight) were randomly selected at 24 h postmortem at a commercial slaughterhouse. 68 69 Whole loin muscle (M. longissimus thoracis et lumborum, LTL) was removed from the left side of each carcass. Loins were cut vertically to a muscle length of 15 cm. Three chops of 70 the medial region of each loin were selected and randomly allocated to three groups: 1) chops 71 72 prepared by cutting vertical to the length of LTL (M-Vertical), 2) chops prepared by cutting vertical to the muscle fiber orientation (F-Vertical), and 3) chops for measuring the loin-eye 73 area and pennation angle of muscle fiber. For the F-Vertical and M-Vertical groups, each 74 75 piece of LTL muscle was divided into 3.0-cm-thickness chops. The LTL chop of the F-Vertical group was used to analyze muscle fiber characteristics, whereas that of the M-76 Vertical group was used to assess muscle fiber characteristics and meat quality. 77

78

79

2.2. Loin-eye area and pennation-angle degree

| 80 | Loin-eye area was measured by tracing the surface of the LTL chop using acetate |
|----|---|
| 81 | paper. The tracings were measured using an Image Pro Plus Program (Media Cybernetics, |
| 82 | Rockville, MD, USA) after scanning by a high-resolution scanner (11000XL, EPSON, |
| 83 | Nagano, Japan). To measure the muscle fiber pennation angle (Fig. 1A), we applied the Kim |
| 84 | et al. (2018) method with some modifications. Briefly, the LTL chop was prepared by cutting |
| 85 | it 2.0 cm in thickness and again parallel to the muscle length. The muscle fiber lean degree to |
| 86 | the LTL muscle fascial membrane was calculated using a protractor. |
| | |

87

88 2.3. Muscle fiber characteristics

Muscle pieces $(1.0 \times 1.0 \times 1.5 \text{ cm})$ were cut from each chop of M-Vertical and F-89 Vertical groups and immediately frozen in 2-methybutane chilled with liquefied nitrogen. 10-90 µm-thickness sections were obtained by making parallel cuts to the chop surface of M-91 Vertical group and by making vertical cuts to the muscle fiber orientation of F-Vertical group, 92 93 respectively, using a cryostat microtome (CM1520, Leica Biosystem, Wtezlar, Germany). The method by Song et al. (2020) with some modifications was used to stain the cross-94 sections and muscle fiber typing. Briefly, each section was incubated with monoclonal anti-95 myosin heavy chain (MHC) antibodies purchased from DSHB (Iowa City, IA, USA), such as 96 BA-F8 (anti-MHC slow/I), SC-71 (anti-MHCs 2a and 2x), BF-35 (anti-MHCs slow/I and 2a), 97 and BF-F3 (anti-MHC 2b). Secondary antibodies (anti-mouse IgG_{2b}, anti-mouse IgG₁, and 98 99 anti-mouse IgM) conjugated with fluorescent dyes (Alexa Fluor 350, 488, 594, and 647; Thermo Fisher Scientific, Waltham, MA, USA) were used. Primary and secondary antibodies 100 were applied to the sections for 1 h at room temperature in a dark container. Images were 101

102 captured using a confocal scanning laser microscope (TCS SP8 STED, Leica Biosystems, Wetzlar, Germany). Three images obtained from the different regions of each section were 103 104 analyzed using Image-Pro Plus 5.1 (Media Cybernetic Inc., Rockville, MD, USA). Based on the specificities of anti-MHC antibodies to MHCs, the muscle fibers were classified as I 105 (MHC I/slow), IIA (MHC 2a), IIX (MHC 2x), and IIB (MHC 2b). Hybrid fibers comprising 106 107 two or more isoforms of MHC were excluded from this study due to their low frequencies. Approximately 500 fibers per sample were typed and their relative fiber compositions 108 (number or area to the total number or area of fibers, respectively, %), cross-sectional area 109 (CSA, µm²), and total number of fibers (TNF) distributed on the loin-eye (chop surface) were 110 analyzed. 111

- 112
- 113 *2.3. Meat quality*

The pH of LTL chop was directly measured via a portable pH meter (Seven2Go, 114 Mettler-Toledo, Greifensee, Switzerland), with triplication into different regions. Meat color 115 on the chop surface was assessed after oxygenation of myoglobin via exposure to air for 30 116 117 min using a colorimeter (CR-400, Minolta Co., Tokyo, Japan). Prior to measuring meat color, the colorimeter was calibrated with a white plate (Y = 93.5, x = 0.3132, y = 0.3198). The 118 color values were expressed by a Commission Internationale de l'Eclairage (CIE, 1978) 119 system, such as lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*). Drip loss was 120 analyzed by suspending the LTL chops in a plastic bag for 24 h at 4°C according to the 121 method of Honikel (1987), with certain modifications. After suspension, the LTL chops were 122 123 weighed and drip loss was presented as a percentage of the initial weight. Cooking loss was measured by cooking the LTL chops in a water bath at 75°C after packing them in a plastic 124 bag. When the internal temperature reached 70°C, the chops were removed from the water 125

bath and cooled to room temperature. Cooking loss was presented as a percentage of the
initial weight of the LTL chop. After measuring cooking loss, the chops were used for
Warner-Bratzler shear force (WBSF) measurements. Three cores (1.3 cm in diameter) were
obtained from each chop by making parallel cuts to the chop surface. The cores were cut
vertically using a texture analyzer TA1 (Ametek, Largo, FL, USA) with a V-shaped blade,
and the WBSF values (N/cm²) were presented as the average of the three cores.

132

133 *2.4. Statistical analysis*

Experimental data obtained from each pork chop are presented as standard error of 134 means (SE). Data was statistically analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA). 135 136 To compare the relative CSA and fiber area composition between the F-Vertical and M-Vertical groups and among the muscle fiber types, t-tests were performed within the same 137 fiber type or within the same group. Correlation coefficients between pennation angle, loin-138 eye area, muscle fiber characteristics, and meat quality traits were analyzed using the CORR 139 procedure. Values of p<0.05, p<0.01, and p<0.0001 were considered as statistically 140 significant. 141

142

143

144 **3. Results**

145 *3.1. Architecture, muscle fiber characteristics, and meat quality of porcine LTL muscle*

Porcine LTL is unipennate type muscle, as illustrated in Figure 1. Due to the leaned muscle fibers, the surface of the loin chop cut vertically to the length of the muscle had ellipse-shaped muscle fibers regardless of the fiber type. Muscle fiber characteristics (relative number, relative area, CSA, and total number of fibers) were evaluated using the cross-

| 150 | section prepared by making a vertical cut to the muscle fiber orientation (F-Vertical) as well |
|-----|---|
| 151 | as by using the section cut vertically to the muscle length (M-Vertical) (Fig. 1B). Their basic |
| 152 | data, loin-eye area, and pennation angle degree are listed in Table 1. The loin-eye area was |
| 153 | 52.90 cm ² . Muscle fibers were leaned at 51.33° to 69.00° from the pennation angle. For the |
| 154 | F-Vertical muscle fiber characteristics, the relative number and area of muscle fiber varied |
| 155 | according to the fiber type, and relatively higher compositions were found in type IIB |
| 156 | (65.97% relative number and 75.10% relative area). Types I and IIA presented numerically |
| 157 | lower compositions of both fiber number and area than those observed in type IIX or IIB. The |
| 158 | CSA of types IIB and IIX was larger than that of type II or IIA. Moreover, the CSA of F- |
| 159 | Vertical (CSA _F) was higher in types IIX or IIB than that in type I or IIA. A similar result was |
| 160 | observed for fiber composition (relative fiber number and area) in M-Vertical when compared |
| 161 | to that in F-Vertical; however, CSA in M-Vertical (CSA _M) was larger than that in F-Vertical, |
| 162 | with approximately twice the size, regardless of the fiber type (Table 1 and Fig. 2). For meat |
| 163 | quality characteristics, a pH range of 5.40–5.62 was observed in pork loin chop. The CIE L*, |
| 164 | CIE a*, and CIE b* on the chop surface were 51.06, 5.51, and 5.62, respectively. Drip loss |
| 165 | was 2.62% with 0.65% of SD, whereas cooking loss was 22.16% with 2.78% of SD. The |
| 166 | WBSF values ranged from 27.58 N/cm ² to 47.34 N/cm ² , as listed in Table 1. |

167

3.2. Relationships between loin-eye area, pennation angle, and muscle fiber characteristics Table 2 presents the results of correlation coefficients between the loin-eye area, pennation angle, and muscle fiber characteristics. Loin-eye area was correlated only with the relative area of type IIB regardless of the cross-section type (r = 0.39, p<0.05 for F-Vertical; r

172 = 0.42, p<0.05 for M-Vertical). Pennation angle revealed negative correlations with CSA_M of

all fiber types (p < 0.05), whereas the other traits of muscle fiber characteristics and loin-eye

| 174 | area were not significantly correlated with the pennation angle ($p>0.05$). TNF was negatively |
|-----|---|
| 175 | correlated with the pennation angle (r = -0.45 , p< 0.05). The relative number of type I was |
| 176 | positively correlated with TNF (p <0.05), whereas that of type IIB presented negative |
| 177 | correlation with TNF (p<0.01) regardless of the cross-section type. Nevertheless, no |
| 178 | significant correlation was found in all compositions of the fiber area in F-Vertical as well as |
| 179 | M-Vertical (p>0.05). CSA_M of all fiber types exhibited negative correlations with TNF |
| 180 | (p<0.01), whereas no significant correlations were observed in any type of muscle fiber in F- |
| 181 | Vertical between TNF - and CSA_F (p>0.05). |
| 182 | |
| 100 | 2.2 Deletionships between loin and more namedian and more lefther about statistics |

3.3. Relationships between loin-eye area, pennation angle, and muscle fiber characteristics on chop surface and meat quality

Among the meat quality traits, pH, CIE L*, and WBSF were significantly correlated 185 with the pennation angle or muscle fiber characteristics (p < 0.05), whereas other meat quality 186 traits such as CIE a*, CIE b*, drip loss, and cooking loss did not indicate significant 187 correlations with the pennation angle or muscle fiber characteristics (p>0.05; Table 3). 188 Moreover, the loin-eye area did not reveal significant correlations with any meat quality traits 189 (p>0.05). pH was negatively correlated with TNF (r = -0.45, p<0.05) but positively 190 correlated with CSA_M of type IIX (r = 0.36, p<0.05) and IIB (r = 0.39, p<0.05) fibers. CIE L* 191 was positively correlated with type IIX in the relative fiber number or relative area (p < 0.05). 192 WBSF was positively correlated with the pennation angle (r = 0.53, p<0.01), relative fiber 193 area (r = 0.55, p<0.1), and CSA_M (r = 0.41, p<0.05) of type IIX; whereas it was negatively 194 195 correlated with the relative fiber area of type IIB (r = -0.55, p<0.01). 196

197

198 4. Discussion

Muscle fibers were leaned at 51.33° to 69.00° in the porcine LTL muscle, which is 199 200 within the range of previous observations in porcine LTL (48.00° to 83.33°) or bovine longissimus lumborum (31.36° to 53.90°) muscles (Kim et al., 2018; Derington et al., 2011). 201 Pennation angle was not significantly correlated with the loin-eye area but was considerably 202 203 correlated with the total number and fiber size on the loin-eye (chop) surface (Table 2). As illustrated in Figure 1B, CSA is dependent on the pennation degree (θ) of muscle fiber, that 204 205 is, LTL with a smaller θ has smaller CSA_M but LTL comprising muscle fiber with bigger θ has larger CSA_M regardless of the original CSA (transverse to the muscle fiber orientation) of 206 the fibers. Consequently, TNF on the loin-eye is negatively correlated with both the pennation 207 208 angle and CSA_M of muscle fibers regardless of the fiber type. Furthermore, a negative correlation between TNF and muscle fiber size was previously demonstrated, and these 209 parameters are considered for muscle development and muscle mass, which are influenced by 210 various factors (species, breed, gender, hormones, genotype, and growth promoters) (Rehfeldt 211 et al., 2000; Rehfeldt and Ender, 1993; Ryu et al., 2004; Ullman and Oldfors, 1989). Both are 212 213 positively correlated with muscle mass, whereas the results observed in this study were not correlated with the loin-eye area. Whether these inconsistent results seem to be a result of the 214 muscle fiber architecture (unipennate type of LTL muscle) and pennation degree remains 215 216 unclear. Further studies on the relationships between TNF, CSA, and muscle mass in LTL muscle by considering the muscle fiber architecture are warranted to understand the muscle 217 218 development of livestock animals in detail.

Muscle fibers exposed to the surface of the loin chop look elliptical and their size is considerably larger than that of the transverse section (Fig. 2A); however, muscle fiber composition (relative area) did not differ between the F-Vertical and M-Vertical. The size and 222 composition relationships in glycolytic fibers (IIX and IIB) with pH and tenderness observed in the present study are in accordance with the findings in previous studies that demonstrated 223 224 their positive or negative correlations with pH or tenderness, respectively, regardless of the animal species, breed, gender, age, and muscle type (Choi and Kim, 2009; Joo et al., 2013; 225 Karlsson et al., 1993; Kim et al., 2013a; Kim et al., 2018; Larzul et al., 1997; Ryu et al., 226 2008). The CIE L* and CIE a* in meat color are highly affected by the fiber size and 227 composition (Hwang et al., 2010; Kim et al., 2010; Kim et al., 2013a; Ozawa et al., 2000), 228 that is, oxidative fibers are positively correlated with CIE a*, whereas glycolytic fibers are 229 negatively correlated with CIE a* but positively correlated with CIE L* due to their diverse 230 reliance on oxygen and consequent demands of myoglobin for energy metabolism of different 231 232 muscle fiber types (Cassens and Cooper, 1971; Kim et al., 2010; Ozawa et al., 2000; Whipple et al., 1992). In this study, type IIX, one of the glycolytic fibers, revealed positive correlations 233 in its relative area and CSA with CIE L*; however, except for these results, we did not find 234 any relationship between the muscle fiber characteristics and color traits. These results are 235 observed due to the difference in shape and size of the muscle fiber cross-section between F-236 237 Vertical and M-Vertical. F-Vertical is a general type of muscle fiber cross-section, whereas M-Vertical is considerably larger and elliptical in shape, as aforementioned. Moreover, the 238 converse relationships of WBSF were observed in relative fiber areas of type IIX (positive) 239 240 and IIB (negative). The fiber area composition and size of IIX revealed a similar relationship to tenderness when compared to the previous observations in beef and pork (Karlsson et al., 241 1993; Renand et al., 2001). The positive correlation between the pennation angle and WBSF 242 243 indicates that the shear force increased because the muscle fiber cross-section came closer to the longitudinally cut shape as the muscle fibers gradually leaned with the increase in the 244 pennation angle. 245

246 **5. Conclusions**

Pennation angle degree determines the muscle fiber shape, size, and total number of f 247 248 ibers on loin eye (chop surface). In particular, muscle fiber size is highly affected by the penn ation angle regardless of the fiber type. Consequently, muscle fiber size and composition of gl 249 ycolytic fibers (IIX and IIB) are closely related to the pH, CIE L*, and tenderness of loin cho 250 p. A high degree of pennation angle and large size of IIX fiber negatively affected the tendern 251 ess of the loin chop. In conclusion, these observations help to understand the porcine LTL mu 252 scle architecture and the influence of muscle fiber characteristics of the chop surface on the m 253 eat quality of pork loin. 254

255 **References**

| 256 | Bee G, Calderini M, Biolley C, Guex G, Herzog W, Lindemann MD. 2007. Changes in the |
|-----|--|
| 257 | histochemical properties and meat quality traits of porcine muscles during the growing- |
| 258 | finishing period as affected by feed restriction, slaughter age, or slaughter weight. J Anim |
| 259 | Sci 85:1030-1045. |
| 260 | Cassens RG, Cooper CC. 1971. Red and white muscle. Adv Food Res 19:1-74. |
| 261 | Chang KC, da Costa N, Blackley R, Southwood O, Evans G, Plastow G, Wood JD, Richardson |
| 262 | RI. 2003. Relationships of myosin heavy chain fibre types to meat quality traits in |
| 263 | traditional and modern pigs. Meat Sci 64:93-103. |
| 264 | Choi YM, Kim BC. 2009. Muscle fiber characteristics, myofibrillar protein isoforms, and meat |
| 265 | quality. Livest Sci 122:105-118. |
| 266 | CIE (Commission Internationale de l'Eclairage). 1978. Reccommendations on uniform color |
| 267 | spaces-color difference equations, Psychometric Color Terms. Supplement No. 2 to CIE |
| 268 | Publication No. 15 (E-1.3.1.). Paris, France: Commission Internationale de l'Eclairage. |
| 269 | Derington AJ, Brooks JC, Garmyn AJ, Thompson LD, Wester DB, Miller MF. 2011. |
| 270 | Relationships of slice shear force and Warner-Bratzler shear force of beef strip loin |
| 271 | steaks as related to the tenderness gradient of the strip loin. Meat Sci 88:203-208. |
| 272 | Honikel KO. 1987. How to measure the water-holding capacity of meat? Recommendation of |
| 273 | standardized methods. In P. V. Tarrant, G. Eikelenboom, & G. Monin (Eds.), Evaluation |
| 274 | and control of meat quality in pigs (pp. 129–142). Dordrecht: Martinus Nijhoof. |
| 275 | Hwang YH, Kim GD, Jeong JY, Hur SJ, Joo ST. 2010. The relationship between mu |
| 276 | scle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korea |
| 277 | n native cattle) steers. Meat Sci 86:456-461. |
| 278 | Joo ST, Kim GD, Hwang YH, Ryu YC. 2013. Control of fresh meat quality through |

279 manipulation of muscle fiber characteristics. Meat Sci 95:828-826.

- Karlsson A, Enfält AC, Essén-Gustavsson B, Lundström K, Rydhmer L, Stern S. 1993. Muscle
 histochemical and biochemical properties in relation to meat quality during selection for
 increased lean tissue growth rate in pigs. J Anim Sci 71:930-938.
- 283 Kim GD, Kim BW, Jeong JY, Hur SJ, Cho IC, Lim HT, Joo ST. 2013a. Relationship of carcass
- weight to muscle fiber characteristics and pork quality of crossbred (Korean native black
 pig × Landrace) F2 pigs. Food Bioprocess Technol 6:522-529.
- Kim GD, Jeong, JY, Hur SJ, Yang, HS, Jeon JT, Joo ST. 2010. The relationship between meat
 color (CIE L* and a*), myoglobin content, and their influence on muscle fiber
 characteristics and pork quality. Korean J Food Sci Anim Resour 30:626-633.
- Kim GD, Overholt MF, Lowell JE, Harsh BN, Klehm BJ, Dilger AC, Boler DD. 2018.
 Evaluation of muscle fiber characteristics based on muscle fiber volume in porcine
 longissimus muscle in relation to pork quality. Meat Muscle Biol 2:362-374.
- Kim GD, Ryu YC, Jeong JY, Yang HS, Joo ST. 2013b. Relationship between pork quality and
 characteristics of muscle fibers classified by the distribution of myosin heavy chain
 isoforms. J Anim Sci 91:5525-5534.
- 295 Larzul C, Lefaucheur L, Ecolan P, Gogue J, Talmant A, Sellier P. 1997. Phenotypic and

296 genetic parameters for longissimus muscle fiber characteristics in relation to growth,

carcass, and meat quality traits in large white pigs. J Anim Sci 75:3126-3137.

Lebret B, Le Roy P, Moniin G, Lefaucheur L, Caritez JC, Talmant A, Elsen JM, Sellier P. 1999.

- Influence of the three RN genotypes on chemical composition, enzyme activities, and myofiber characteristics of porcine skeletal muscle. J Anim Sci 77:1482-1489.
- 301 Lefaucheur L, Ecolan P, Plantard L, Gueguen N. 2002. New insights into muscle fiber types in

the pig. J Histochem Cytochem 50:719-730.

| 303 | MacIntosh BR, Gardiner PF, McComas A. 2005. Muscle architecture and muscle fiber anatomy. |
|-----|--|
| 304 | In Skeletal muscle form and function (pp. 3-21). USA: Human Kinetics. |
| 305 | McDonald KS, Wolff MR, Moss RL. 1997. Sarcomere length dependence of the rate of |
| 306 | tension redevelopment and submaximal tension in rat and rabbit skinned skeletal muscle |
| 307 | fibers. J Physiol 501:607-621. |
| 308 | Narici M. 1999. Human skeletal muscle architecture studied in vivo by non-invasive imaging |
| 309 | techniques: Junctional significance and application. J Electromyogr Kines 9:97-103. |
| 310 | Nigg BM, Herzog W. 1994. Biomechanics of the musculoskeletal system. Chichester: John |
| 311 | Wiley & Sons. |
| 312 | Ozawa S, Mitsuhashi T, Mitsumoto M, Matsumoto S, Itoh N, Itagaki K, Kohno Y, Dohgo Y. |
| 313 | 2000. The characteristics of muscle fiber types of longissimus thoracis muscle and their |
| 314 | influences on the quantity and quality of meat from Japanese Black steers. Meat Sci. 54:65- |
| 315 | 70. |
| 316 | Rehfeldt C, Ender K. 1993. Skeletal muscle cellularity and histochemistry in response to |
| 317 | porcine somatotrophin in finishing pigs. Meat Sci 34:107-118. |
| 318 | Rehfeldt C, Fiedler I, Dietl G, Ender K. 2000. Myogenesis and postnatal skeletal muscle cell |
| 319 | growth as influenced by selection. Livest Prod Sci 66:177-188. |
| 320 | Renand G, Picard B, Touraille C, Berge P, Lepetit J. 2001. Relationships between muscle |
| 321 | characteristics and meat quality traits of young Charolais bulls. Meat Sci 59:49-60. |
| 322 | Ryu YC, Choi YM, Lee SH, Shin HG, Choe JH, Kim JM, Hong KC, Kim BC. 2008. |
| 323 | Comparing the histochemical characteristics and meat quality traits of different pig |
| 324 | breeds. Meat Sci 80:363-369. |
| 325 | Ryu YC, Kim BC. 2006. Comparison of histochemical characteristics in various pork groups |
| 326 | categorized by postmortem metabolic rate and pork quality. J Anim Sci 84:894-901. |
| | 15 |

- Ryu YC, Rhee MS, Kim BC. 2004. Estimation of correlation coefficients between histological
 parameters and carcass traits of pig longissimus dorsi muscle. Asian-Aust J Anim Sci
 17:428-433.
- 330 Song S, Ahn CH, Kim GD. 2020. Muscle fiber typing in bovine and porcine skeletal muscles
- using immunofluorescence with monoclonal antibodies specific to myosin heavy chain
- isoforms. Food Sci Anim Resour 40:132-144.
- Ullman M, Oldfors A. 1989. Effects of growth hormone on skeletal muscle. I. Studies on
 normal adult rats. Acta Physiol Scand 135:531-536.
- 335 Whipple G, Hunt MC, Klemm RD, Kropf DH, Goodband RD, Schricker BR. 1992 Effects of
- porcine somatotropin and supplemental lysine on porcine muscle histochemistry. J Muscle
- 337 Foods 3:217-227.

338 Figure Legends

Fig. 1. Schematic representation of pork loin (M. longissimus thoracis et lumborum) 339 architecture and stained cross-sections. (A) Pork loin cut parallel to the muscle length and 340 image of the chop surface cut vertical to the muscle length. Pennation angle (θ), learning degree 341 of muscle fiber to the fascia of loin muscle; arrows with broken line, muscle fiber orientation. 342 (B) Schematic representation of muscle fiber and cross-sections: F-Vertical, cut vertical to the 343 muscle fiber orientation; M-Vertical, cut vertical to the muscle length. CSAF, cross-sectional 344 345 area of F-Vertical; CSA_M, cross-sectional area of M-Vertical. (C) Cross-sections stained with anti-myosin heavy chain (MHC) antibodies. Images were presented after merging four images 346 obtained from different anti-MHC antibodies (BA-F8, SC-71, BF-35, and BF-F3; DSHB, IA, 347 USA). Muscle fiber types were distinguished with different colors (I, pink; IIA, yellowish green; 348 IIX, green; IIB, blue). Bar = $100 \mu m$. 349

350

Fig. 2. Comparison of muscle fiber size and relative fiber area between the different crosssectional cuts and muscle fiber types. Relative cross-sectional areas (CSA) (A) and relative fiber area (B) were compared for cross-sections between F-Vertical (cut vertical to the muscle fiber orientation) and M-Vertical (cut vertical to the muscle length) and among the muscle fiber types. Different letters on the bar indicate significant differences between F-Vertical and M-Vertical groups (x, y) within the same fiber type or among different muscle fiber types (a–c) within the same cross-sectional group at p<0.05.

| | Variables | Mean | SD | Min | Max | |
|---------------------------------|---|-------|----------|---------|---------|----------|
| Pennation an | gle (°) | 60.44 | 4.42 | 51.33 | 69.00 | |
| Loin-eye area | $a (cm^2)$ | 52.90 | 4.93 | 45.16 | 58.00 | |
| Muscle fiber | characteristics | | | | | |
| | | Ι | 10.30 | 2.73 | 5.19 | 17.21 |
| | Relative fiber number (%) | IIA | 9.59 | 3.19 | 4.87 | 16.56 |
| | Relative fiber fidfiber (%) | IIX | 13.72 | 3.14 | 9.42 | 20.45 |
| | | IIB | 65.97 | 5.32 | 53.90 | 75.00 |
| | | Ι | 6.42 | 1.60 | 3.99 | 9.50 |
| <i>F-Vertical</i> ¹⁾ | Relative fiber area (%) | IIA | 5.46 | 1.97 | 2.51 | 9.43 |
| r-venicui | Relative liber area (%) | IIX | 12.86 | 3.58 | 7.09 | 20.98 |
| | | IIB | 75.10 | 4.50 | 66.83 | 83.48 |
| | | Ι | 2976.77 | 604.90 | 2078.12 | 4222.43 |
| | Cross-sectional area (μm^2) | IIA | 2708.69 | 523.81 | 1952.74 | 3810.24 |
| | | IIX | 4438.99 | 728.74 | 3372.31 | 5770.76 |
| | | IIB | 5489.73 | 755.18 | 4340.15 | 7172.77 |
| | Total number of fiber ($\times 10^6$ | 5) | 0.60 | 0.12 | 0.41 | 0.83 |
| | | Ι | 11.91 | 1.65 | 4.83 | 16.08 |
| | $\mathbf{P}_{\mathbf{q}}$ | IIA | 8.72 | 1.53 | 5.11 | 13.22 |
| | Relative fiber number (%) | IIX | 15.48 | 2.28 | 11.52 | 19.81 |
| | | IIB | 63.17 | 4.37 | 54.10 | 72.94 |
| | | Ι | 6.37 | 1.78 | 3.12 | 9.75 |
| <i>M-Vertical</i> ²⁾ | ⁾ Relative fiber area (%) | IIA | 5.53 | 2.23 | 2.11 | 10.18 |
| | Relative liber area (%) | IIX | 13.01 | 4.35 | 7.13 | 24.56 |
| | | IIB | 75.08 | 5.59 | 61.54 | 85.72 |
| | | Ι | 6310.86 | 1631.94 | 3781.78 | 9300.70 |
| | Cross-sectional area (µm ²) | IIA | 5643.51 | 1350.22 | 3910.83 | 8493.19 |
| | Cross-sectional area (µm) | IIX | 9247.79 | 2314.12 | 6136.95 | 15991.19 |
| | | IIB | 11427.02 | 2248.79 | 8050.47 | 16079.25 |
| Meat quality | | | | | | |
| pН | | | 5.58 | 0.06 | 5.40 | 5.62 |
| | CIE L* | | 51.06 | 2.44 | 46.22 | 55.84 |
| Meat color | CIE a* | 5.51 | 0.80 | 4.45 | 7.14 | |
| | CIE b* | | 5.62 | 1.74 | 3.26 | 8.87 |
| Drip loss (%) | | | 2.62 | 0.65 | 1.42 | 3.54 |
| Cooking loss | | 22.16 | 2.78 | 16.34 | 27.94 | |
| Warner-Bratz | zler shear force (N/cm ²) | 33.49 | 5.47 | 27.58 | 47.34 | |

Table 1. Basic data (mean, SD, min, and max) of porcine longissimus thoracis et lumborum 358 muscles 359

¹⁾ Cross-sections prepared by cutting vertical to the muscle length.

360

| Variables | | Loin-eye area | Pennation angle | TNF ¹⁾ |
|-----------------------------------|-----|------------------|-----------------|-------------------|
| Pennation angle | | | | -0.45* |
| Loin-eye area | | | 0.19 | 0.05 |
| <i>F</i> -Vertical ²) | | | | |
| | Ι | -0.29 | -0.18 | 0.49** |
| Relative number | IIA | -0.03 | -0.03 | 0.07 |
| Relative number | IIX | -0.27 | 0.17 | 0.24 |
| | IIB | 0.07 | 0.15 | -0.56** |
| | Ι | -0.18 | 0.03 | 0.24 |
| D - 1 - 4' | IIA | 0.00 | -0.23 | 0.30 |
| Relative area | IIX | -0.21 | 0.30 | 0.09 |
| | IIB | 0.39* | 0.04 | -0.32 |
| | Ι | -0.18 | 0.03 | 0.24 |
| Crease as officer all area | IIA | 0.00 | -0.23 | 0.31 |
| Cross-sectional area | IIX | -0.21 | -0.21 0.30 | |
| | IIB | 0.32 0.04 | | -0.32 |
| M-Vertical ³⁾ | | | | |
| | Ι | -0.25 | -0.08 | 0.41* |
| Relative number | IIA | 0.05 | 0.02 | 0.14 |
| Kelative number | IIX | -0.16 | 0.16 | 0.29 |
| | IIB | 0.13 | 0.17 | -0.51** |
| | Ι | -0.26 | 0.00 | 0.31 |
| Relative area | IIA | -0.11 | -0.17 | 0.15 |
| Kelative alea | IIX | -0.33 | 0.24 | -0.06 |
| | IIB | 0.42* | -0.11 | -0.12 |
| | Ι | -0.09 | 0.55** | -0.78*** |
| Cross-sectional area | IIA | -0.32 | 0.51* | -0.71** |
| Cross-sectional area | IIX | -0.27 | 0.68** | -0.79*** |
| | IIB | 0.14 | 0.57** | -0.83*** |

Table 2. Correlation coefficients between loin-eye area, pennation angle, and muscle fiber characteristics of pork loin chops

¹⁾ Total number of fiber on chop surface of pork loin.

²⁾ Cross-sections prepared by cut vertical to the muscle fiber orientation.

³⁾ Cross-sections prepared by cut vertical to the muscle length.

*, p<0.05; **, p<0.01; ***, p<0.0001.

| Variables | | pН | ľ | Meat colo | r | - Drip loss | Cooking loss | WBSF ¹⁾ |
|-----------------------|-----|--------|--------|-----------|--------|-------------|-----------------|--------------------|
| | | рп | CIE L* | CIE a* | CIE b* | | | |
| Pennation angle | | 0.09 | 0.16 | 0.32 | 0.14 | 0.18 | -0.10 | 0.53** |
| Loin-eye area | | -0.10 | 0.16 | 0.19 | 0.06 | -0.22 | -0.10 | 0.07 |
| Total number of fiber | | -0.45* | 0.12 | -0.20 | 0.18 | -0.09 | 0.16 | -0.33 |
| | Ι | -0.11 | -0.15 | -0.06 | 0.02 | -0.06 | 0.27 | -0.02 |
| Relative fiber | IIA | 0.14 | 0.12 | 0.08 | 0.08 | 0.19 | 0.30 | 0.31 |
| number | IIX | -0.02 | 0.44* | -0.18 | 0.15 | 0.09 | 0.31 | 0.11 |
| | IIB | 0.07 | -0.14 | 0.28 | 0.00 | 0.07 | -0.24 | -0.21 |
| | Ι | -0.20 | -0.22 | -0.05 | -0.09 | -0.17 | 0.10 | 0.00 |
| Relative fiber | IIA | 0.00 | -0.10 | 0.05 | 0.08 | 0.03 | 0.23 | 0.29 |
| area | IIX | 0.31 | 0.35* | 0.04 | 0.10 | 0.27 | 0.30 | 0.55** |
| | IIB | -0.18 | -0.18 | -0.04 | -0.08 | -0.17 | -0.32 | -0.55** |
| | Ι | 0.30 | -0.31 | -0.26 | -0.31 | -0.23 | -0.04 | 0.25 |
| Cross-sectional | IIA | 0.31 | -0.12 | -0.01 | -0.09 | 0.09 | -0.19 | 0.18 |
| area | IIX | 0.36* | -0.08 | -0.02 | -0.19 | 0.10 | 0.02 | 0.41* |
| | IIB | 0.39* | -0.18 | -0.07 | -0.32 | -0.26 | -0.14 | 0.24 |

Table 3. Correlation coefficients between loin-eye area, pennation angle, muscle fiber characteristics on chop surface of pork loin, and meat quality characteristics

¹⁾ Warner-Bratzler shear force.

*, p<0.05; **, p<0.01.





