# **Food Science of Animal Resources**

Food Sci. Anim. Resour. 2023 May 43(3):531~539 DOI https://doi.org/10.5851/kosfa.2023.e15

#### ARTICLE



# Objective Meat Quality from Quality Grade and Backfat Thickness of Hanwoo Steers

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Received	January 16, 2023
Revised	March 10, 2023
Accepted	March 30, 2023

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Zhen Song https://orcid.org/0000-0002-4388-3873 Inho Hwang https://orcid.org/0000-0002-2474-2733 Abstract The objective of this study was to determine the effects of quality grade (QG), and back-fat thickness on the carcass traits and meat quality properties of Hanwoo steers. Fifty carcasses were sorted into two QG (QG 1+ and 1) and three back-fat thickness (<10 mm, 10 to 19 mm and  $\geq$ 19 mm) groups. After investigating the carcass traits (rib eye, back-fat thickness, weight, color, yield index, maturity, marbling score, and texture), the longissimus lumborum muscles from the carcass groups were collected and analyzed for meat quality (pH, color, cooking loss, and moisture), texture profiles [Warner-Bratzler shear force (WBSF), and tensile tests], and fatty acid. Results showed that marbling score (p<0.001), moisture (p<0.05) and tensile tests values (p<0.05) had a significant differences between QG1+ and QG1. No differences in pH, color traits, cooking loss and WBSF values occurred between the QG groups. Regarding the back-fat thickness effect, we observed that the carcass weight, yield index (p < 0.001), yield grade (p < 0.001) and marbling score (p<0.05) had a significant differences among the back-fat thickness groups. Regarding the meat quality, moisture content and WBSF values (p<0.01) among the back-fat thickness groups. The back-fat thickness did not affect the pH, color, cooking loss and tensile tests. The QG and back-fat thickness did not affect the fatty acids contents (p>0.05). It may be concluded that the carcass traits and meat quality were significantly affected the QG and back-fat thickness.

Keywords texture, quality traits, fatty acid, back-fat thickness, quality grade

# Introduction

Hanwoo is major beef cattle breed which is the most preferable by Korean consumers due to its unique flavor and juiciness (Kim and Lee, 2003). The Korean carcass grading system determines the quality grade (QG) of cattle carcass based on its marbling score (MS), along with other carcass characteristics, such as meat color, fat color, texture, and maturity (Cho et al., 2010). At the beginning (1990s), the Korean carcass grading system contained 3 QGs (QG1, QG2, and QG3), and 5 beef marbling standard (BMS) from 1 (low marbling) to 5 (high marbling). An additional QG (QG 1+), and two MS (No. 6 and 7) were introduced in 1997. In 2004, the QG 1++, and MS No. 8 and No. 9

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were further introduced. Nowadays, beef with the QG 1++ and 1+ are considered the highest-quality products in Korean meat market. Previous study had proved that the BMS and QG were more strongly related to beef price (Beak et al., 2021).

It is known that adipose tissues may depot in subcutaneous, visceral, intermuscular and intramuscular areas in cattle carcass. In addition to acting as a system for storing excess energy and protecting the body against dangers, body fat also contributes to fat combustion. Furthermore, adipokines are also produced by body fat, and can affect a wide range of physiological functions, such as appetite, fertility, neuronal development, inflammation responses, and insulin action (Ouchi et al., 2011). The excessive deposition of subcutaneous and intermuscular fat, however, is also shown to negatively impact the quality of carcass and cause financial losses rather than improving the quality of meat. According to Tatum et al. (1980), subcutaneous fat thickness may serve as an addition to or a replacement for the current strategy (maturity, marbling, and QG) of forecasting the palatability of beef. Additionally, it was discovered that back-fat thickness and tenderness had been found to be correlated to each other in beef (Lochner et al., 1980). Although much of the research has focused on marbling content (QG or marbling grade), Back-fat thickness has several advantages over QG or marbling grade, including being easier to apply, more uniform, and more easily related to slaughter cattle grades. Therefore, the goal of this study was to ascertain how Hanwoo steer carcass features and meat quality characteristics were affected by QG and back-fat thickness.

### **Materials and Methods**

#### **Carcass selection**

Total of fifty Hanwoo steers from a commercial abattoir were used in the present investigation. They were slaughtered at the ages from 28 to 30 months (GQ1+ slaughtered average age at 29.33 months, GQ1 slaughtered average age at 29.1 months, back-fat thickness <10 mm slaughtered average age at 28.88 months, back-fat thickness 10 to 19 mm slaughtered average age at 29.47 months, back-fat thickness  $\geq$ 19 mm slaughtered average age at 28.88), carcasses were graded and analyzed for carcass traits according to the Ministry of Government Legislation (MOLEG, 2014) by an official grader. Measurements were made between the last rib and the first lumbar vertebrae to determine the thickness of back-fat and the rib eye area. Based on the Grading Livestock Products Criteria of MOLEG (2014) criteria, the marbling grade ranges from 1 to 9 (from trace to plentiful), the fat color ranges from 1 to 7 (from white to yellow), the meat color ranges from 1 to 7 (from light cherry red to dark red), the texture ranges from 1 to 5 (from fine to coarse), and the maturity ranges from 1 to 9 (from young to old). The yield index, which was a function of the carcass weight, the rib eye area, and the back-fat thickness, was used to evaluate the yield grade, as follows: Yield index =  $[68.184 - \{0.625 \times \text{Back-fat thickness (mm)}\}] + \{0.130 \times \text{Rib eye area}\}$  $(cm^2)$  - {0.024 × Carcass weight (kg)} + 3.23. A yield index value  $\geq$ 67.20 is indicative of yield grade 1, yield index value between 63.30 to 67.20 is indicative of yield grade 2, and yield index value ≤63.30 is indicative of yield grade 3. Based on the results of grading evaluation, the carcasses were grouped into QGs 1+(n=26; marbling is 6 to 7) and 1(n=22; marbling is4 to 5). The carcasses were also classified into three: low (<10 mm, n=8), medium (10 to 19 mm, n=34) and high ( $\geq$ 19 mm, n=8) back-fat thickness groups.

#### Sample collection

All the *longissimus lumborum* (LL) muscles were collected from the left sides of carcasses, and analyzed for meat quality. Muscles were sealed in vacuum packaged and aged at 4°C for 3 days. When the aging was completed, sample was taken out from the chilling room and made into sub-sample sizes depending on each analysis. The samples for fatty acid were vacuumpackaged and store at -80°C until use.

#### pH and color measurements

The pH values of these samples were determined using a Meat pH meter (HI99163, Hanna Instrument, Padova, Italy) and analyzed using the method of Ji (Ji et al., 2010). Each sample was analyzed 4 times.

A Konica Minolta Spectrophotometer (CM-2500d, Konica Minolta, Osaka, Japan), equipped with an 8-mm measuring port AT, D65 illuminant, and a 10° observer, was used to determine the color of the meat. Each sample was measured on three different surface locations after 30 min of blooming at 4°C. CIE L\*, CIE a\*, and CIE b\* were as the color attributes.

#### Cooking loss and texture profile

Cooking loss, and texture analysis [Warner-Bratzler shear force (WBSF) and Tensile tests] were following by the method of Ba et al. (2014), all the samples were determined with 3-cm thick steak and with weight of 300 g (n=2 per sample). The samples were put into plastic bags and cooked in a water bath until their inner temperature reached 70°C. An iron constantan thermocouple with a probe inserted into the samples' geometric centers was used to measure the temperature. After cooking, samples were cooled in circulating water for 30 minutes. The following equation was used to determine cooking loss on samples by weighing them before and after cooking:

Cook loss (%) = 
$$\frac{\text{Weight of uncooked sample (g)} - \text{Weight of cooked sample (g)}}{\text{Weight of uncooked sample (g)}} \times 100$$
(1)

After cooking loss measurement, each sample was made into strips (sample for WBSF was evaluated with 1.5 cm diameter; sample for tensile test with 50×10×10 mm thick) parallel to the fiber direction. WBSF was measured at crosshead speed of 400 mm/min using a 50 kfg load cell of Instron Universal Testing Machine (Model 3342, Instron, Norwood, MA, USA). The tensile test was measured at a 50 mm/min stretching speed using the same device.

#### **Moisture content**

The moisture content in the samples was measured using a Halogen moisture analyzer (HR73, Mettler-Toledo, Schwerzenbach, Switzerland). Before using, the device was adjusted its moisture meter to zero. The minced sample (2.5 g each) were put in aluminum dish, hypodispersion and dried at 105°C. After the program of the moisture analyzer finished, the values were read on the display of the machine.

#### Fatty acid profiles

The fatty acid composition was extracted and analyzed using the procedure developed by Rule (1997). The meat sample was cut into thin slices and freeze-dried for 48 hours before being ground up. Then, an HPLC grade methanol solution (2 mL) and 14% boron-trifluoride in methanol (2 mL, BDH, BDH Laboratory Supplies, Poole, England) were both added to a 20 mL headspace container together with 500 mg of the dried sample. The vials were placed on a heating block set at 80°C with a tight cap on them. The samples were remained at this temperature for 2 h with 5 min interval vortex mixing. After cooling, 3 mL distilled water and 3 mL hexane were added. Following the centrifugation at 1,000×g for 5 min, 1 mL of the upper hexane phase was transferred to test vials. Fatty acid composition was determined using a Gas Chromatography (GC,

7890B)-Mass Spectrometer system (MS, 5977B; Agilent, Santa Clara, CA, USA) with an auto-sampler. A split ratio of 50:1 was used with a carrier gas speed of 45 cm/s and an injector temperature of 250°C. A WCOT-fused silica capillary column (30 m×0.25 mm×0.25 µm) with a 1.0 mL/min helium flow was used to separate fatty acid methyl esters. The oven was set up to do the following: 150°C/2 min, 150°C to 230°C at 10°C/min, 230°C/15 min. By comparing the retention times of the fatty acids to those of standard fatty acids, the fatty acids were identified (F.A.M.E. Mix., CRM 18918, 47015-U, Sigma-Aldrich, St. Louis, MO, USA). The proportion of the fatty acid calculated use the peak area of each identified fatty acid against total identified peak area. Every sample was test 2 times.

#### **Statistical analysis**

All data were analyzed using the General Linear Model Procedure of the SAS version 9.3 program (SAS Institute, Cary, NC, USA; SAS, 2011). The QG and back-fat thickness were considered as the fixed factors while the carcass traits, quality attributes, etc. were considered as the variables. The Duncan's Multiple Range Test was used to compare means. The significance level was set at p<0.05.

#### **Results and Discussion**

#### Effect on carcass traits

The carcass traits from the QG and back-fat groups are presented in Table 1. It was observed that the traits (yield traits, back-fat thickness, rib eye, carcass weight, maturity, colors and texture) were not significantly different among the groups. However, the QG1+ generally had heavier carcasses, thicker back-fat, higher yield grade, and larger rib eye area compared to the QG1. Thus, better QG score had a larger rib eye, this can be attributed to rib eye area that had highly positive correlations with slaughter weight and marbling (do Nascimento et al., 2022). QG1+ also had higher maturity, lower red meat color, and lower white fat color. This was in agreement with that reported in previous study (Park et al., 2002). The QG 1+ also exhibited a lower texture scores compared to the QG1. The QG affected marbling (p<0.001) between different groups. The data support the finding that the QG had a stronger relationship with the MS than other scores, thus, the QG had the strongest relationship with the MS of carcasses (Park et al., 2002).

In our study, all the animals were slaughtered at the close age, but a high variation in back-fat thickness was found, indicating that the deposition of adipose tissues varies among them. This means that the beef cattle might have intense developmental changes (hyperplasia and hypertrophy) in adipose tissue during growth (Cianzio et al., 1985), and lipid metabolism (Frylinck et al., 2013). The carcass weight increased with increased back-fat thickness (p<0.05). Additionally, the yield grade increased as increasing the back-fat thickness. These views concur with the points that in the meat, lipid accumulation is directly related to the yield of meat, especially in subcutaneous adipose tissues (Bresolin et al., 2022). The marbling and rib eye area increased with increased back-fat thickness, which agrees with finding of Moon et al. (2003). Previous studies proved that intramuscular fat content is closely related to the subcutaneous fat content (Malheiros et al., 2020; May et al., 1992). But the back-fat thickness ( $\geq$ 19 mm) showed decreased. Results also discordant with the viewpoint that maturity was inversely proportional to color and texture (Moon et al., 2006). There may be two reasons for this difference, one is the gender is different, previous study used cows and we used steer, the other is the fat thickness is different, the fat thickness is around 10 mm in previous study, but in our study the back-fat thickness from 7.5 mm to 22.13 mm. Results proved that excessive deposition of subcutaneous does not contribute to meat quality, and could detract the

Traits	QG1+	QG1	SEM	F-value	BK1	BK2	BK3	SEM	F-value
Number	26.00	22.00			8.00	34.00	8.00		
Back-fat (mm)	14.82	13.62	4.84	0.72	7.50°	14.00 <sup>b</sup>	22.13ª	4.92	70.61***
Rib eye (cm <sup>2</sup> )	88.11	87.05	9.14	0.16	85.88	88.47	84.50	9.20	0.71
Carcass weight (kg)	428.26	413.00	47.50	1.23	401.25 <sup>b</sup>	422.06 <sup>ab</sup>	445.25ª	47.38	1.79
Yield index <sup>1)</sup>	63.33	64.30	3.65	0.84	68.26ª	64.03b	57.88°	3.68	50.62***
Yield grade <sup>2)</sup>	2.44	2.10	0.71	2.95	1.25°	2.44 <sup>b</sup>	3.00 <sup>a</sup>	0.72	26.29***
Maturity <sup>3)</sup>	2.19	2.14	0.38	0.15	2.25	2.16	2.13	0.38	0.25
Marbling <sup>4)</sup>	6.41ª	4.57 <sup>b</sup>	1.05	157.07***	4.75 <sup>b</sup>	5.78 <sup>a</sup>	5.63ª	1.05	3.24*
Meat color <sup>5)</sup>	4.89	5.14	0.62	2.03	5.38	4.94	5.00	0.64	1.56
Fat color <sup>6)</sup>	2.96	3.05	0.29	0.99	3.13	2.97	3.00	0.29	0.91
Texture <sup>7)</sup>	1.04	1.14	0.28	1.72	1.25	1.06	1.13	0.31	1.21
Month	29.33	29.10	1.65	0.24	28.88	29.47	28.88	1.61	0.72

Table 1. Carcass traits of Hanwoo steers as affected by back-fat thickness and quality grades

<sup>1)</sup> Yield index =  $[68.184 - \{0.625 \times \text{Back-fat thickness (mm)}\}] + \{0.130 \times \text{Rib eye area (cm}^2)\} - \{0.024 \times \text{Carcass weight (kg)}\} + 3.23$ .

<sup>2)</sup> Yield grade was scored as: Yield grade 1 means the value of yield index  $\geq$ 67.20; yield grade 2 means the value of yield index between 63.30 to 67.20, and yield grade 3 means the value of yield index  $\leq$ 63.30.

<sup>3)</sup> Maturity ranges from youthful to mature.

<sup>4)</sup> Marbling grade ranges from 1 to 9 (from trace to plentiful).

<sup>5)</sup> Meat color ranges from 1 to 7 (from light cherry red to dark red).

<sup>6)</sup> Fat color ranges from 1 to 7 (from white to yellow).

<sup>7)</sup> Texture ranges from 1 to 5 (from fine to coarse).

<sup>a-c</sup> Signifies that different superscripts in the same row are significantly different.

\* p<0.05, \*\*\* p<0.001.

QG1+, quality grade1+; QG1, quality grade1; BK1, back-fat thickness <10 mm; BK2, back-fat thickness 10 to 19 mm; BK3, back-fat thickness  $\geq$ 19 mm.

carcass quality.

#### Effect on meat quality traits

The effects of QG and back-fat thickness on the pH, color, moisture, cooking loss and texture properties (WBSF and tensile tests) of Hanwoo LL muscles are presented in Table 2. Results of the QG groups showed that moisture (p<0.05) and tensile tests values (p<0.05) had a significant differences between QG1+ and QG1. No differences in pH, color traits, cooking loss and WBSF values occurred between the QG groups. Results of the back-fat thickness groups showed that moisture and WBSF values (p<0.05) had a significant differences among different back-fat thickness groups. No differences in pH, color traits, cooking loss and tensile tests values occurred between the QG groups.

The QG and back-fat thickness did not affect the pH. Result is in agreement with Kim and Lee (2003), who found no difference in pH values among QG groups of Hanwoo steers. Similarly, the QG and back-fat thickness did not affect all the color traits. This may be due to the similar ultimate pH values in all the treatment groups because pH is known as a factor influencing the denaturation of pigment proteins.

Cooking loss values showed no difference in QG and back-fat thickness groups, but QG1+ had lower values than QG1 group. This agrees with the findings of Yim et al. (2015). Ozawa et al. (2000) studied on Japanese black steer meat and also found that cooking loss is negatively correlated with QG.

The QG and back-fat thickness both affected the moisture content significantly (p<0.05). The moisture decreased with increased QG or back-fat thickness. Yim et al. (2015) also found that moisture content is significantly lower in high-QG beef.

Traits	QG1+	QG1	SEM	F-value	BK1	BK2	BK3	SEM	F-value
pH	5.50	5.50	0.05	0.09	5.48	5.50	5.50	0.04	1.23
CIE L*	39.84	39.29	2.49	0.57	39.04	39.82	39.47	2.44	0.34
CIE a*	19.30	19.54	1.82	0.20	19.00	19.51	19.05	1.82	0.38
CIE b*	14.96	14.73	2.38	0.11	14.79	14.64	14.82	2.28	0.03
Cooking loss (%)	15.24	15.57	2.43	0.21	15.79	15.32	15.36	2.48	0.12
Moisture (%)	56.06 <sup>b</sup>	59.02ª	4.32	6.15*	58.92ª	57.57 <sup>ab</sup>	54.77 <sup>b</sup>	4.32	2.08
WBSF (kgf)	3.03	3.42	0.70	3.90	3.88 <sup>a</sup>	3.12 <sup>b</sup>	3.05 <sup>b</sup>	0.67	5.25**
Maximum load (kgf)	2.36 <sup>b</sup>	2.74 <sup>a</sup>	0.65	4.24*	2.92	2.49	2.50	0.62	1.59
Tensile strain (%)	144.80 <sup>b</sup>	184.50ª	63.83	$4.96^{*}$	190.34	154.79	184.47	62.72	1.49
Tensile extension (mm)	14.60 <sup>b</sup>	18.74ª	6.55	5.12*	19.14	15.38	18.87	6.44	1.49

Table 2. Effects of back-fat and quality grade on meat characteristic of LL muscle

<sup>a,b</sup> Signifies that two superscripts in the same row are significantly different.

\* p<0.05, \*\* p<0.01.

LL, *longissimus lumborum*; QG1+, quality grade1+; QG1, quality grade1; BK1, back-fat thickness <10 mm; BK2, back-fat thickness 10 to 19 mm; BK3, back-fat thickness  $\geq$ 19 mm; WBSF, Warner-Bratzler shear force.

But, the highest level of back-fat thickness had the intermediate values of marbling, meanwhile, has the lowest moisture in this study, this suggested that back-fat thickness may be more related with moisture than marbling.

In QG groups, WBSF values were lower in the higher QG (QG 1+). This may be related to adipocytes excessive development caused by disorganization of the perimysial connective tissue (Malheiros et al., 2020). In the different back-fat thickness groups, results showed that the WBSF values decreased as increasing the back-fat thickness. This is also in agreement with the finding that the back-fat may improve tenderness by reducing cold-shortening, thick back-fat may insulate the carcass and lead to slow postmortem chilling (Dolezal et al., 1982; Malheiros et al., 2020; May et al., 1992). But the effect of decreased WBSF value is inconspicuous when the back-fat over 19 mm. This also agrees with the finding of Dolezal et al. (1982), who reported that WBSF values decreased rapidly as back-fat increasing from 2.53 mm to 12.69 mm, when the back-fat from 12.7 mm to 17.77 mm, the effect of decrease WBSF value is inconspicuous. But when the back-fat over 17.78 mm the WBSF value increase instead of decrease. May et al. (1992) also found that sensory tenderness scores decreased (p<0.05) for steaks from steers with a mean back-fat thickness of 21.08 mm. Previous study suggested that the carcass maturity (Jerez-Timaure et al., 2013) and age could affect tenderness (Kang et al., 2022). Maturity and age were kept similar for all the animals in our study. Therefore, could be due to the effects of back-fat thickness.

Tensile tests values (p<0.05) had a significant difference between QG. This may be related to QG1+ had higher marbling. But the tensile tests values had no difference in different back-fat thickness groups. Results showed that WBSF and tensile tests had the same trend in QG groups or back-fat thickness groups, but the significant differences were different in QG groups or back-fat thickness groups. The reason is may be that the tensile test determines the meat's strength in the direction of the fibers. The myofibres' single internal components can convey the ensuing stress without the help of their membranes or connective tissue. There are no confounding effects, such as friction or a change in sample orientation under load.

#### Fatty acid profiles

Fatty acids influence the flavor release of meat during cooking (Frank et al., 2016). It is generally known that phospholipids and unsaturated lipids play a role in the formation of volatile aromas (Elmore et al., 1999). The effects of QG and back-fat

thickness on fatty acid profile in LL muscles are shown in Table 3. A total of 11 fatty acids (FA) were identified, in which the most predominant FAs were oleic acid (C18:1, 42.78% to 43.1%), palmitic acid (C16:0, 20.83% to 21.21%) and stearic acid (C18:0, 13.24% to 13.76%). Our results are consistent with those previously reported for Hanwoo cattle beef (Lee et al., 2010).

The QG and back-fat thickness did not affect the individual fatty acids as well as total saturated fatty acid, mono-and polyunsaturated fatty acid content (p>0.05). Although, Legako et al. (2015) have reported that C18:1 and MUFA were positively associated with beef flavor. PUFA and C18:2 might be negatively associated with beef flavor (Piao et al., 2019). In our study, the QG for QG1+ and QG1, the quality were quality near, thus, the values had no statistical difference. Meanwhile, the  $\geq$ 19 mm back-fat thickness group had the highest values of C18:1 and MUFA. Results proved that Hanwoo beef from the back-fat thickness over 19 mm may have higher flavor.

# Conclusion

In summary, the Hanwoo carcasses with a better QG (QG1+) had a higher carcass weight, MS, and yield grade. However, the QG1+ group exhibited lower moisture and cooking loss, as well as lower values for the WBSF and tensile tests.

Back-fat thickness was found to significantly affect meat tenderness; increasing back-fat thickness resulted in a decrease in WBSF value, but excessive deposition of back-fat (over 19 mm) had a negative effect on the tensile test value.

# **Conflicts of Interest**

The authors declare no potential conflicts of interest.

Fatty acid	QG1+	QG1	SEM	F-value	BK1	BK2	BK3	SEM	F-value
C10:0 (Capric)	0.14	0.14	0.04	0.05	0.15	0.14	0.15	0.03	0.51
C12:0 (Lauric)	0.24	0.19	0.14	1.46	0.21	0.22	0.21	0.14	0.03
C14:0 (Myristic)	5.75	5.51	0.74	1.25	5.64	5.63	5.77	0.73	0.12
C16:0 (Palmitic)	20.85	21.21	1.21	1.01	20.83	21.04	20.85	1.16	0.16
C16:1 (Palmitoleic)	8.80	8.34	1.53	1.26	8.23	8.71	9.26	1.38	1.14
C18:0 (Stearic)	13.66	13.76	1.09	0.09	13.63	13.72	13.24	1.09	0.63
C18:1 (Oleic)	43.10	43.00	1.87	0.05	42.78	42.96	43.06	1.83	0.05
C18:2 (Linoleic)	6.42	6.87	1.37	1.24	7.35	6.58	6.46	1.34	1.23
C18:3 (g-Linoleic)	0.65	0.55	0.22	2.22	0.74	0.59	0.58	0.22	1.55
C20:0 (Arachidic)	0.41	0.41	0.12	0.00	0.45	0.41	0.40	0.12	0.38
C24:0 (Lignocerate)	0.02	0.01	0.05	0.6	0.00	0.01	0.02	0.04	0.45
SFA	41.07	41.23	2.04	0.06	40.92	41.17	40.63	2.01	0.46
PUFA	7.07	7.42	1.43	0.69	8.09	7.17	7.04	1.62	1.36
MUFA	51.90	51.34	2.04	0.71	51.01	51.67	52.32	2.02	0.69

Table 3. Fatty acid content of LL muscle subjected to different levels of back-fat thickness and quality grades

As stated in the Materials and Methods section, the individual fatty acid content per meat (g/100 g meat) was computed.

LL, *longissimus lumborum*; QG1+, quality grade1+; QG1, quality grade1; BK1, back-fat thickness <10 mm; BK2, back-fat thickness 10 to 19 mm; BK3, back-fat thickness  $\geq$ 19 mm; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids.

# **Acknowledgements**

It should be acknowledged that this work was supported by the Research Program for the Animal Molecular Genetics & Breeding Center (Project No. PJ01316904), Rural Development Administration, Korea.

### **Author Contributions**

Conceptualization: Hwang I. Data curation: Song Z. Formal analysis: Song Z, Hwang I. Methodology: Song Z. Software: Song Z, Hwang I. Validation: Song Z. Investigation: Song Z. Writing - original draft: Song Z. Writing - review & editing: Song Z, Hwang I.

### **Ethics Approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.

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