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Sensory Quality and Histochemical Characteristics of *Longissimus Thoracis* Muscles between Hanwoo and Holstein Steers from Different Quality Grades

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Abstract This study compared the meat quality characteristics, palatability, and histochemical characteristics of low-marbled Hanwoo and Holstein steers of different beef quality grades (1, 2, and 3). No differences were observed in muscle pH_{24 h} and cooking loss between the groups ($p > 0.05$); however, quality grade 1 of Hanwoo steers (HA1) showed a darker muscle surface compared to grade 1 of Holstein steers (HO1) (30.9 vs. 33.9, $p < 0.05$). The HA2 group exhibited a lower value of Warner-Bratzler shear force compared to the HO1 and HO3 groups (60.8 vs. 69.2 and 87.8 N, $p < 0.001$). For sensory quality attributes, steaks from the HA1 group showed higher scores of softness, initial tenderness, and amount of perceptible residue than steaks from the HO1 group ($p < 0.001$). Within the quality grade 2, Hanwoo steers had a higher score of softness compared to Holstein steers ($p < 0.001$). There were no differences in juiciness and flavor intensity between Hanwoo and Holstein steers at the same quality grade ($p > 0.05$). This difference in tenderness attributes between the breeds within the quality grade was associated with morphological traits of muscle bundle, and Hanwoo steers had smaller bundle area (0.37 vs. 0.50 mm², $p < 0.05$) and higher fiber number per bundle (88.2 vs. 121, $p < 0.05$) compared to Holstein steers. Therefore, bundle characteristics of *longissimus thoracis* muscle can be crucial for explaining factor for the explanation of tenderness variations between different breeds at the same beef quality grade or marbling.

Keywords sensory quality, muscle bundle, Hanwoo, Holstein, beef quality grade

Introduction

It is well known that, among the sensory quality characteristics of cooked beef, tenderness is a critical determinant of overall palatability (Hulánková et al., 2018; Miller et al., 2001), and it largely contributes to the satisfaction, dissatisfaction, and purchase-related decision of consumers (Choi and Kim, 2009; Lee et al., 2019). In response, the meat industry has been making efforts to improve tenderness and produces beef with a consistent tenderness (Anderson et al., 2012). In contrast, beef

palatability is primarily the interactive result of various genetic and environmental factors, such as breed, gender, age, muscle type, and feeding regime (Hulánková et al., 2018; Koohmaraie et al., 2002; Lee et al., 2018). Among these factors, cattle breed is an important determinant to cause variations in tenderness, as significant differences were observed in the intramuscular fat (IMF) contents and histochemical characteristics of skeletal muscles from the different breeds (Albrecht et al., 2006). In general, Korean consumers tend to prefer beef with higher beef marbling standard (BMS) scores, since high-marbled beef exhibited greater acceptability of tenderness, juiciness, and flavor compared to those of low-marbled beef (Lee and Choi, 2019). Primarily, the Hanwoo breed has the potential to deposit a higher degree of marbling compared to the other cattle breeds, such as the Holstein breed (Lee et al., 2019), and exhibited a greater appearance rate of 1⁺⁺ and 1⁺ grades than the other meat-type breeds (48.0% vs. 3.3%) according to the carcass grading standard of Korean Institute of Animal Products Quality Evaluation (KAPE, 2021).

Within the quality grade or BMS score, differences in the palatability can exist between the Hanwoo and the other meat-type breeds due to the other factors influencing tenderness (Lee et al., 2018). The morphological and metabolic characteristics of skeletal muscle can also be an essential factor in determining the organoleptic characteristics of cooked beef (Choi and Kim, 2009). Choi et al. (2019) reported that muscle bundle area was negatively correlated with the sensory tenderness attributes conducted by the trained panelists. Moreover, Angus, known as a typical beef breed, had a smaller bundle area (Albrecht et al., 2006), and exhibited a lower Warner-Bratzler shear force (WBS) compared to double-muscling Belgian blue, known as an extreme breed for muscle growth (Wheeler et al., 2001). Thus, muscle bundle characteristics are associated with the variations in sensory tenderness, as muscle bundle, a group of muscle fibers, is responsible for maintaining the surface stiffness in living and postmortem muscles (Lee et al., 2018; Schleip et al., 2006).

On the other hand, there is only limited information about the cause of differences in the sensory quality characteristics between the Hanwoo and Holstein steers of the same quality grade. Therefore, the objectives of this study were to compare the meat quality, and palatability characteristics of low-marbled Hanwoo and Holstein steers of different quality grades. Moreover, this study investigated the histochemical characteristics of *longissimus thoracis* (LT) muscle to establish the cause of differences in tenderness between the low-marbled Hanwoo and Holstein breeds within the quality grade.

Materials and Methods

Animals and muscle samples

Fifty-nine steers (35 Hanwoo [quality grade 1, n=23; quality grade 2, n=12] and 24 Holstein [quality grade 1, n=8; quality grade 2, n=10; quality grade 3, n=6]) were obtained in six batches (11–12 Hanwoo steers per day and 8 Holstein steers per day). Approximately 25 g of muscle samples was taken for the histochemical analysis from the LT muscle at the 12–13th thoracic vertebrae, and immediately frozen in liquid nitrogen, and stored at –80°C.

At 24 h postmortem, the carcasses from Hanwoo and Holstein steers were graded following the carcass quality standards of the KAPE (2021). The KAPE provided the age at slaughter, carcass weight, loin-eye area, back-fat thickness, marbling scores, and beef quality grades of each carcass. However, quality grade 3 of Hanwoo steers was excluded from all the statistical analyses because data were obtained from only two steers. After quality grading, muscle chunks were dissected between the 9–13th thoracic vertebrae, and the meat quality measurements were immediately conducted. For the eating quality evaluation, each muscle chunk was cut into steak-sizes (1.5 cm thickness; approximately 120 g), and then stored at –25°C.

Beef quality measurements

Muscle pH was assessed after carcass quality grading using a portable pH meter (Testo 206-pH2, Testo AG, Lenzkirch, Germany). After 30 min of blooming at 4°C in a cold room, the meat surface color was measured using a chromameter (CR-400, Minolta Camera, Osaka, Japan), and values were expressed as lightness (L*), redness (a*), and yellowness (b*) (CIE, 1978). To measure the water-holding capacity, drip loss, and cooking loss were measured according to the previously reported procedure described by Honikel (1998). After cooking loss measurement, the same samples were used for the WBS analysis. Each meat sample was cut into more than 8 cores (1.27 cm diameter), and the force values were measured using an Instron Universal Testing Machine (Model 1011, Instron, Norwood, MA, USA) mounted with a Warner-Bratzler blade operating at a crosshead speed of 200 mm/min (AMSA, 1995).

Sensory quality evaluation

Sensory quality analysis was conducted using 59 meat samples from Hanwoo and Holstein steers during 12 sessions (4–5 beef loins per session). Ten trained panels (five females and five males; ages 24–46 years) were used in this study. Approval was granted by the Kyungpook National University (KNU) Bioethics Committee (protocol number 2019-0027). Sensory panel training was performed for at least 6 mon at the Muscle Biology Laboratory of KNU according to the guidelines of AMSA (1995). Sensory evaluation was performed based on previously reported procedure (Lee and Choi, 2019; Lee et al., 2019). The frozen samples were thawed at 4°C for 18 h. Beef steaks were cooked by pan-frying with turn-over every 3 min, until the core temperature reached 71°C using an induction range (CIR-IH300RGL, Cuchen, Cheonan, Korea). Trained panelists evaluated the sensory quality characteristics of cooked beef loins, including five tenderness attributes, juiciness, flavor intensity, off-flavor intensity, and mouth coating, using a 9-point scale.

Histochemical analysis

Cross-sections (10 µm thickness) of muscle block (1.2×1.2×2 cm) were continuously obtained using a cryostat CM1860 (Leica, Germany) at –25°C. Muscle sections from each sample were stained with hematoxylin and eosin (Cardiff et al., 2014). Mean fiber area, total fiber number, bundle area, fiber number per bundle, and total bundle number were calculated (Lee and Choi, 2019). Muscle bundle area and fiber number per bundle were observed at 40× magnifications, and more than 30 bundles were measured for each sample. Total fiber number and total bundle number were calculated by dividing the loin-eye area.

Statistical analysis

The general linear model procedure was performed using SAS[®] software (SAS Institute, Cary, NC, USA) to compare meat quality, sensory quality, and histochemical characteristics between Hanwoo and Holstein steers of different quality grades. Significant differences in the least square means (LSM) among the groups were compared by the probability difference option at $p < 0.05$. All data were presented as LSM with standard error.

Results

Comparison of marbling score and meat quality characteristics between Hanwoo and Holstein steers

Table 1 shows the results of meat quality characteristics of the bovine LT muscle in each group. As expected, the marbling

Table 1. Comparison of marbling score and meat quality characteristics between Hanwoo and Holstein steers of different beef quality grades

Breed	Hanwoo		Holstein			Level of significance
	1 (n=23)	2 (n=12)	1 (n=8)	2 (n=10)	3 (n=6)	
Quality grade						
Marbling score	4.57 ^a (0.10) ¹⁾	2.42 ^b (0.13)	4.75 ^a (0.16)	2.10 ^b (0.14)	1.00 ^c (0.18)	***
Muscle pH _{24h}	5.53 (0.03)	5.52 (0.04)	5.62 (0.05)	5.58 (0.04)	5.61 (0.06)	NS
Meat color						
Lightness (L*)	30.9 ^{bc} (0.68)	29.6 ^c (0.94)	33.9 ^a (1.16)	32.7 ^{ab} (1.04)	33.1 ^a (1.34)	*
Redness (a*)	16.4 (0.48)	16.4 (0.67)	17.9 (0.82)	17.5 (0.73)	18.9 (0.94)	NS
Yellowness (b*)	8.32 (0.49)	7.55 (0.68)	8.46 (0.84)	7.94 (0.75)	8.66 (0.97)	NS
Water holding capacity						
Drip loss (%)	0.95 ^b (0.12)	1.43 ^a (0.16)	0.61 ^b (0.20)	0.81 ^b (0.18)	0.59 ^b (0.23)	*
Cooking loss (%)	23.5 (0.85)	23.8 (1.18)	19.7 (1.44)	20.4 (1.29)	21.4 (1.66)	NS
Warner-Bratzler shear force (N)	56.6 ^d (1.68)	60.8 ^d (2.43)	69.2 ^c (2.90)	78.7 ^b (2.43)	87.8 ^a (3.44)	***

¹⁾SEM.

^{a-d} Different superscripts in the same row significant differences ($p < 0.05$).

* $p < 0.05$, *** $p < 0.001$; NS, not significant.

score was clearly different depending on the quality grades regardless of breeds ($p < 0.001$). No significant difference was detected in muscle pH_{24h} among the groups ($p > 0.05$). For the meat color, Holstein steers exhibited a higher lightness value compared to Hanwoo steers ($p < 0.05$), although there was no difference between the HA1 and HO2 groups (30.9 vs. 32.7, $p > 0.05$). Redness and yellowness values did not significantly differ among the groups ($p > 0.05$). Although samples from the HA2 group exhibited a higher drip loss compared to samples from the other groups ($p < 0.05$), cooking loss was not different among all groups ($p > 0.05$). There was no difference in WBS value within the Hanwoo group ($p > 0.05$); whereas a marked difference was observed between the quality grades in Holstein steers ($p < 0.001$).

Comparison of sensory quality characteristics between Hanwoo and Holstein steers

Fig. 1 displays sensory quality characteristics of cooked beef from each group evaluated by trained panelists. From all tenderness attributes, statistically significant differences were found among the groups ($p < 0.001$). Softness and initial tenderness scores decreased as the quality grades decreased within Hanwoo or Holstein steers, and softness score tended to be lower in the Holstein group than in the Hanwoo group within the same grade ($p < 0.001$). However, quality grade 2 samples of Hanwoo and Holstein steers showed a similar score in initial tenderness (5.10 vs. 5.06, $p > 0.05$). There were no differences in chewiness and rate of breakdown between the Hanwoo and Holstein steaks within the same grade ($p > 0.05$), although significant differences were observed among the quality grades within the breed ($p < 0.001$). No difference was observed in the amount of perceptible residue between the HO2 and HA2 groups (4.86 vs. 5.22, $p > 0.05$). In Holstein steers, a significant difference was detected in juiciness score among the grades, and the HO1 group exhibited a higher juiciness compared to the

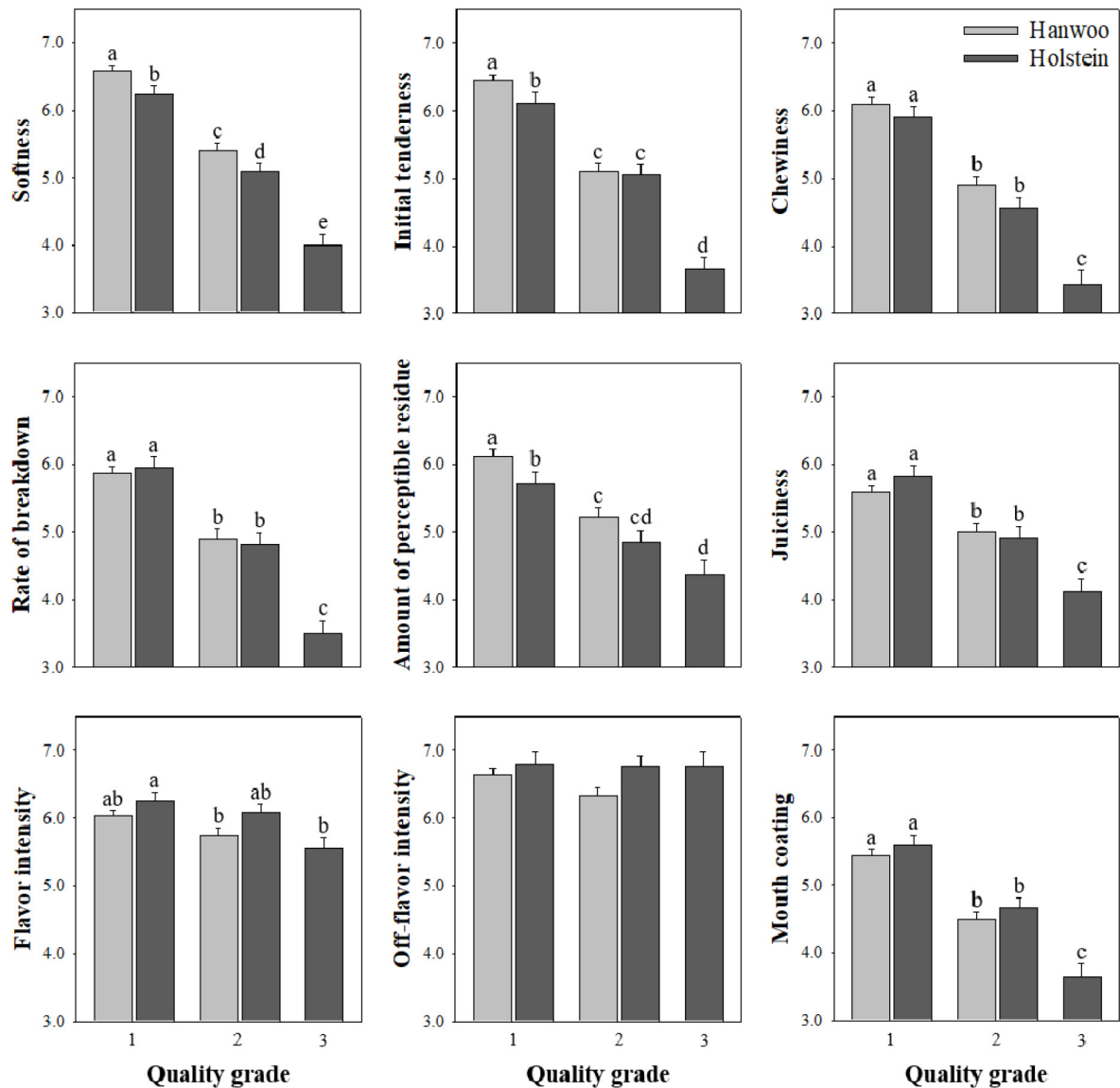


Fig. 1. Comparison of sensory quality characteristics between Hanwoo and Holstein steers of different beef quality grades. Score distribution: low to high (1–9); softness: hard to soft; initial tenderness: tough to tender; chewiness: very chewy to very tender; rate of breakdown: very slow to very fast; amount of perceptible residue: abundant to none; juiciness: not juicy to extremely juicy; flavor intensity: very weak to very strong; off-flavor intensity: very strong to very weak; mouth coating: none to very high. Bars indicate standard errors of least-square means. ^{a–e} Different letters represent significant differences ($p < 0.05$).

other grades ($p < 0.001$). The HA2 group had a similar flavor intensity compared to the other groups ($p > 0.05$) except the HO1 group (5.75 vs. 6.25, $p < 0.01$), and there was no difference in off-flavor intensity among the groups ($p > 0.05$). Mouth coating score significantly differed between the quality grades within Hanwoo or Holstein breeds ($p < 0.001$).

Comparison of histochemical characteristics between Hanwoo and Holstein steers

The histochemical characteristics of Hanwoo and Holstein steers from each quality grade are shown in Fig. 2. No significant difference was observed in muscle fiber area among the groups ($p > 0.05$). Due to smaller loin-eye area data not shown and similar fiber size, the Holstein steers exhibited a fewer total fiber number compared to the Hanwoo steers

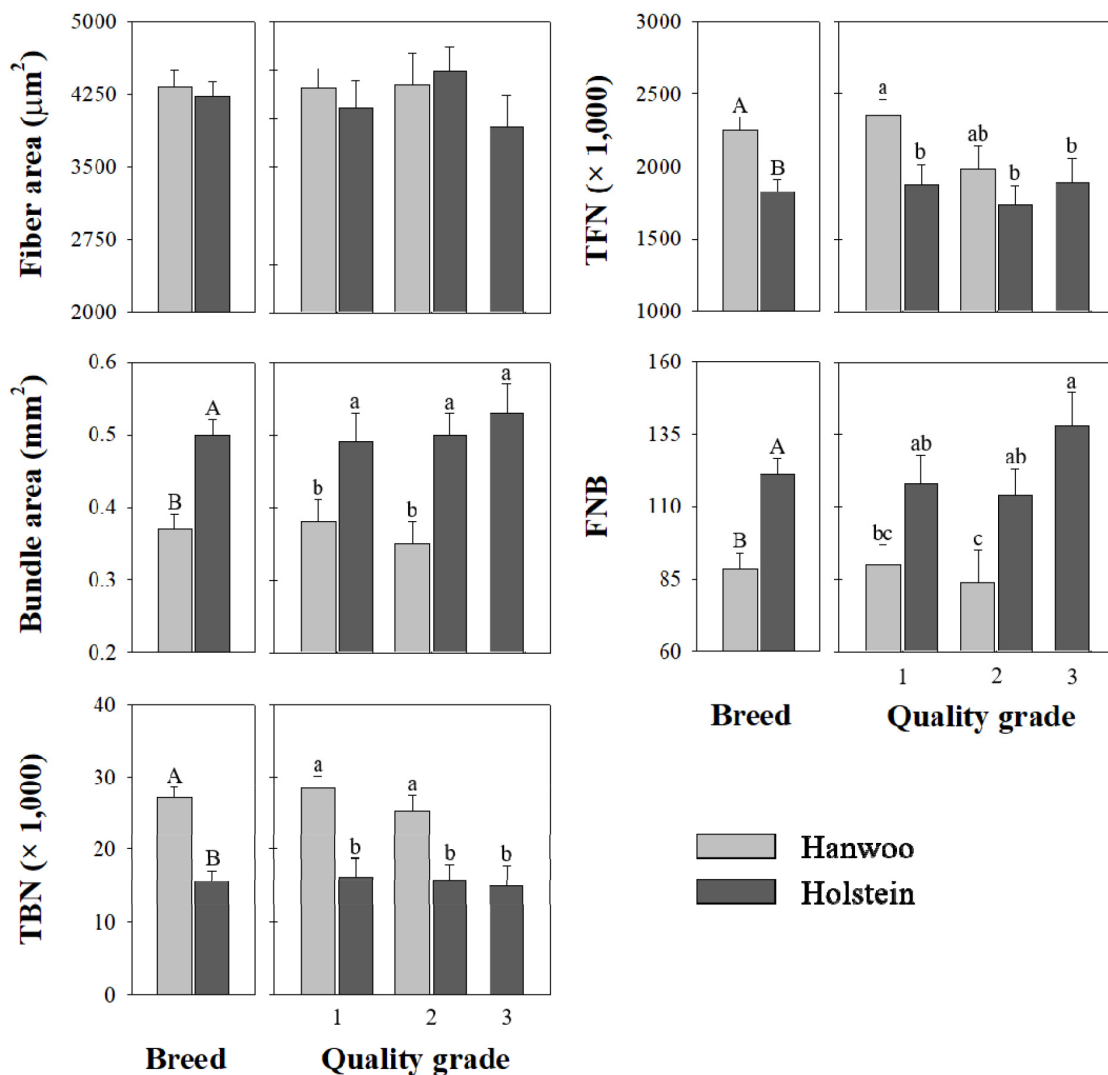


Fig. 2. Comparison of histochemical characteristics between Hanwoo and Holstein steers of different beef quality grades. Bars indicate standard errors of least square means. ^{A,B} Different capital letters indicate significant differences between the breeds ($p < 0.05$). ^{a-c} Different small letters indicated significant differences among the beef quality grades from different breeds ($p < 0.05$). TFN, total fiber number; FNB, fiber number per bundle; TBN, total bundle number.

($p < 0.01$). However, the HA2 group had a similar number compared to the Holstein group ($p > 0.05$). For the muscle bundle characteristics, Holstein steers showed a greater bundle area compared to Hanwoo steers ($p < 0.001$). Higher fiber number per bundle was observed in the HO3 group compared to the HA1 and HA2 groups (138 vs. 89.9 and 83.7, $p < 0.001$). In contrast to the bundle area, the total bundle number was lower in the Holstein group compared to the Hanwoo group ($p < 0.001$).

Discussion

Beef quality grade is an objective evaluation for sorting a heterogeneous population into homogeneous groups based on their organoleptic beef characteristics. A crucial factor in determining the quality grade is the degree of marbling at the standard site (LT muscle surface at the 13th thoracic vertebra) for carcass grading (Lee et al., 2019). Generally, the Hanwoo breed applies a long-term fattening regime to achieve a higher marbling score; thus, it was reported to have greater quality

grades compared to those of the other breeds in Korea (KAPE, 2021; Lee et al., 2019). In contrast, Holstein cattle have a significantly lower IMF content in the LT muscle compared to beef breeds, since dairy breeds are typically selected based on their milk production ability rather than muscle mass and meat quality (Rezagholivand et al., 2021). At the same live weight, Holstein cattle have a lower ratio of carcass weight and poor muscularity compared to beef breeds due to a higher proportion of non-carcass parts, including liver, heart, and kidney (Bown et al., 2016). Moreover, these steers often exhibited a thinner back-fat compared to Angus steers, although the marbling score was similar between the two breeds at the same live weight and at the same maturity level (Muir et al., 2000). Compared with Hanwoo and Holstein steers in this study, carcasses derived from Hanwoo steers had greater loin-eye area and back-fat thickness compared to carcasses derived from Holstein steers at approximately similar carcass weight ($p < 0.001$, data not shown). For meat quality traits, Hanwoo steers exhibited a darker muscle surface compared to Holstein steers ($p < 0.05$), and no difference was observed in cooking loss between carcasses from the two breeds ($p > 0.05$). Bown et al. (2016) compared the meat quality traits between Holstein, Hereford, and Hereford×Holstein steers at the same age, and who reported no breed difference in WBS level. Furthermore, no difference was reported in WBS level among Holstein select, Holstein choice, and Charolais cross-bred choice following the USA Department of Agriculture grades (Schaefer et al., 1986). Interestingly, in this study, at the same quality grade, steaks from Hanwoo steers showed a lower WBS level compared to steaks from Holstein steers ($p < 0.001$). Thus, breed differences were somewhat existed in the carcass and meat quality characteristics between Hanwoo and Holstein steers in this study.

In tenderness attributes, within the quality grade 1, the Hanwoo group exhibited greater scores of softness, initial tenderness, and amount of perceptible residue compared to the Holstein group ($p < 0.001$). A higher softness score was also observed in Hanwoo steers compared to Holstein steers at the quality grade 2 ($p < 0.001$), although the other tenderness attributes were not different between the two breeds ($p > 0.05$). Armbruster et al. (1983) suggested that loin steaks from Angus steers required lower force for penetration and fewer chews before swallowing compared to loin steaks from Holstein steers. However, no cattle breed effect was observed by taste panel in tenderness between Holstein and the other breeds, including Hereford, Charolais, and Jersey (Bown et al., 2016; Schaefer et al., 1986). In contrast, there were generally no differences in juiciness, flavor, and off-flavor between beef and dairy breeds when compared at the same marbling degree (Bown et al., 2016; Schaefer et al., 1986). These results support the findings of the present study. There were no significant differences in juiciness and flavor intensity between two breeds within the same quality grade ($p > 0.05$). Altogether, steaks from typical beef breeds could be tender compared to steaks from dairy breeds, although no breed effects could be observed in the other sensory quality traits at the same quality grade or marbling degree.

Muscle bundle and perimysium play an essential role in load and stress bearing functions under various conditions, and are therefore associated with the integrity of contraction and relaxation of living muscles (Gillies and Lieber, 2011; Schleip et al., 2006). It is well known that the bundle characteristics, especially bundle size and fiber number per bundle, are the cause of variations in the texture feature and firmness of muscle surface during the postmortem periods (Lee et al., 2018). On the other hand, a clear difference was observed in fiber number per bundle or bundle size between the cattle breeds (Albrecht et al., 2013; Norman, 1982). Norman (1982) reported that the Charolais breed harboring a smaller bundle area showed a lower WBS value compared to the Nelore breed harboring a greater bundle area. Within the quality grade, Hanwoo steers harboring a greater fiber number per bundle exhibited visually coarser texture and less tender meat than Hanwoo steers harboring a lower number per bundle (Lee et al., 2018). These findings on the effects of bundle characteristics agree well with the results of this study. Steaks from Hanwoo steers showing a smaller bundle area exhibited a lower required force for the initial few chewing than steaks from Holstein steers showing a larger bundle area within the quality grade ($p < 0.05$).

Conclusion

When comparing the same beef quality grade or marbling score, steaks from Hanwoo steers could be tenderer than steaks from Holstein. This palatability of Hanwoo beef, which can be distinguished from Holstein, was influenced by the muscle bundle characteristics.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Kim EJ, Choi YM. Data curation: Kim JY, Lee B, Choi YM. Formal analysis: Kim JY, Lee B. Methodology: Lee B, Choi YM. Software: Kim JY. Investigation: Kim JY, Lee B. Writing - original draft: Kim JY, Lee B, Kim DH, Lee K, Kim EJ, Choi YM. Writing - review & editing: Kim JY, Lee B, Kim DH, Lee K, Kim EJ, Choi YM.

Ethics Approval

The human ethics approval was granted by the Bioethics Committee of KNU (protocol number: 2019-0027).

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