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ARTICLE

# Comparison of Marbling Fleck Characteristics and Objective Tenderness Parameters with Different Marbling Coarseness within *Longissimus thoracis* Muscle of High-marbled Hanwoo Steer

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**Abstract** It is important to understand how marbling traits and tenderness differ among beef steaks from the carcass grading site and other regions within the *longissimus thoracis* (LT) muscle, as these characteristics are closely associated with consumer acceptability and willingness to purchase. Thus, the aim of this study was to compare the marbling fleck traits and objective tenderness parameters in the groups classified by the coarseness index (CI) of marbling fleck (high and low groups) at the carcass grading site ($13^{th}$ thoracic vertebra; 13T) and three different locations (13T, 9T, and 6T) within the LT muscle from well-marbled (marbling score 7 to 9) Hanwoo steer. Image analysis showed that the longitudinal locations had a significant effect on marbling fleck traits. The total area of large marbling fleck divided by the total marbling area (coarseness) was higher at the central region (13T to 12T) compared to the front thoracic region (6T to 5T) in the high CI group (0.23 vs. 0.17, $p<0.05$), whereas no significant differences were observed in the total number of marbling fleck within the LT muscle in the high or low CI groups ($p>0.05$). Location effect on objective tenderness parameters within the LT muscle was somewhat limited, although the high CI group had a lower Warner-Bratzler shear force (WBS) value than did the low group ($p<0.05$). Taken together, the degree of coarseness of marbling fleck decreased from the carcass grading site to the front thoracic site, whereas the objective tenderness parameters, including WBS and hardness, of the grading site did not differ from the other regions within the LT muscle.

**Keywords** marbling fleck, coarseness, tenderness parameter, Hanwoo

## Introduction

Marbling is the term used to describe the appearance of white flecks or streaks of intramuscular fat (IMF) between muscle bundles (Hocquette et al., 2010). Significant differences between animals, breeds, and muscle types exist not only in the degree of marbling, but also in the morphological characteristics of marbling fleck measured by computerized image analysis (Albrecht et al., 2006; Konarska et al., 2017). These differences can make variations in sensory quality characteristics, as the IMF is one of the major factors that influences palatability of cooked beef, especially tenderness (Wood et al., 2008). Conversely, Gerrard et al. (1996) suggested that the marbling scoring is often overestimated owing to the presence of larger marbling flecks, and consumers do not prefer beef loin with coarse marbling flecks. Thus, marbling fleck traits, such as coarseness and fineness of marbling fleck, can influence palatability and consumer purchase decisions.

Tenderness of cooked beef is a characteristic that varies considerably between muscles. Rhee et al. (2004) reported that the *longissimus* muscle had a lower Warner-Bratzler shear force (WBS) value compared to the *semimembranosus* (SM) and *adductor* muscles. WBS values also differed depending on the location within the muscle, and considerable variation detected in the SM (Rhee et al., 2004) and *biceps femoris* (Searls et al., 2005) muscles. Within the *longissimus* muscle, steak from the lumbar region (sirloin cut; 5$^{th}$ to 1$^{st}$ lumbar vertebrae) was more tender compared to steak from the central region (loin cut; 13$^{th}$ to 10$^{th}$ thoracic vertebrae) (Janz et al., 2006). Additionally, image analysis revealed variations in marbling fleck traits within the *longissimus* muscle of Japanese Black steers (Nakahashi et al., 2007). However, differences in marbling traits and WBS value within the *longissimus thoracis* (LT) muscle of Hanwoo steer have not been elucidated. It is necessary to understand how marbling fleck traits and tenderness differ between beef loins from the carcass quality grading site and other sites in order to improve predictions regarding the quality grading system and satisfaction of consumers. Thus, in this study, muscle samples from high-marbled Hanwoo steer were classified into either low and high coarseness index (CI) groups based on the degree of coarseness of marbling fleck at the carcass quality grading site (13$^{th}$ thoracic vertebra of LT muscle) to compare the carcass traits between the CI groups. We then compared marbling fleck characteristics and objective tenderness parameters between different locations within the LT muscle with different coarseness of marbling fleck.

## Materials and Methods

### Animals and muscle samples

A total of 42 loin cuts from three different locations within the LT muscle of 14 high-marbled Hanwoo steers (aged 27–30 mon; mean carcass weight of 446.1±31.5 kg) were used in this study. The steers were transported to a commercial slaughterhouse, and slaughtered under the same handling conditions. At 24 h postmortem, the carcasses were graded into five quality grades by trained evaluators of the Korea Institute of Animal Products Quality Evaluation (KAPE, 2017). After quality grading, the KAPE provided carcass weight, loin-eye area, back-fat thickness, and marbling score. In this study, carcasses were randomly selected based on their marbling score (seven to nine) in three batches (4 to 5 cattle per day). The LT muscle from the left side of each carcass was dissected between the 13$^{th}$ to 5$^{th}$ thoracic vertebrae, before muscle samples were collected from the central (13$^{th}$ to 12$^{th}$ thoracic vertebra, 13T to 12T), thoracic (9T to 8T), and front thoracic (6T to 5T) regions. Marbling scores for each location were determined at the 13T, 9T, and 6T thoracic vertebrae of LT muscle according to the marbling standard (one to nine; devoid to very abundant) by an official grader. Immediately after, photographs of the dissected muscles were taken with a mirror-type digital camera (shutter speed 1/50 and f-number 3.5; D3200, Nikon Co., Japan) at each loin region for image analysis of marbling flecks. A strobe (Nikon Co., Japan) was used from an angle of 45° from the cut surface to prevent irregular reflection on the muscle surface. Muscle samples were cut into 2.0 cm thick steaks, and the IMF content, cooking loss, WBS, and texture-profile analysis (TPA) were determined. The IMF content was analyzed by the Soxhlet extraction method (AOAC, 2012).

## Image analysis for marbling fleck characteristics

Computerized image analysis for marbling fleck traits on the images of the dissected muscle at three locations was performed using the Beef Analyzer G software (Hayasaka Ricoh Co. Ltd., Japan) developed by Kuchida et al. (2006). The digital color image at the loin cut surface (Fig. 1A) was binarized into muscle (black) and marbling flecks (white; Fig. 1B). In the binarized image, the number and area of marbling fleck were clearly visible. The binarized image was then separated into two particle forms of bigger (>0.5 $cm^2$) and smaller (0.01 to 0.5 $cm^2$) marbling flecks (Fig. 1C and 1D). Marbling fleck measures (Konarska et al., 2017; Kuchida et al., 2006) included the marbling area, percentage of marbling area, number of marbling particle, fineness (F), coarseness (C), and F/C ratio. The smallest particle (<0.01 $cm^2$) was not included for the image analysis, as this particle may appear due to light influx. Thus, marbling area occupied by marbling particles (≥0.01 $cm^2$) was calculated, and the total number of marbling fleck was counted. Marbling area percentage was determined as the proportion of total marbling area divided by the loin-eye area. Fineness index was calculated as the number of small particle per loin-eye area ($cm^2$). Coarseness index (CI) was calculated from the total area of bigger marbling fleck ($cm^2$) divided by the total marbling area ($cm^2$), and the F/C ratio was calculated as the ratio of fineness divided by coarseness.

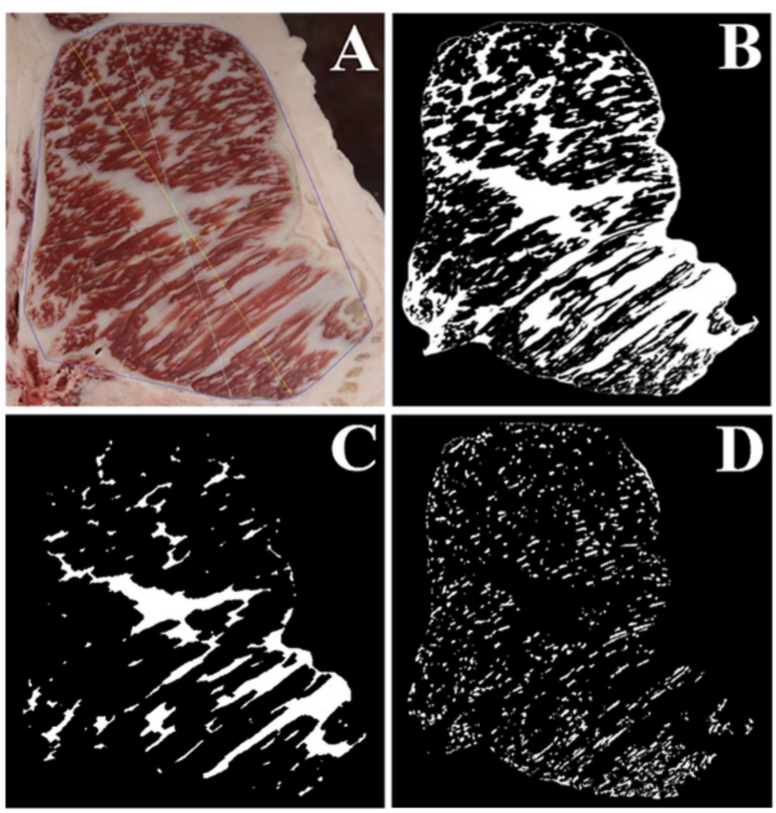


**Fig. 1. Details of image analysis to calculate the characteristics of marbling fleck.** Digital color image (A) of the *longissimus thoracis* muscle cross-section was binarized into muscle and marbling flecks (B) using the image analysis program. The binarized image was separated into two images of bigger (C) and smaller (D) marbling flecks. The fineness was calculated from the number of smaller marbling flecks (0.01 to 0.5 $cm^2$) per loin-eye area, and the coarseness was calculated by division of the total area of bigger marbling flecks (above 0.5 $cm^2$) by the total marbling area measured.

### Cooking loss and objective texture parameters

For cooking loss measurement, beef loin samples were cut and weighed (approximately 70 to 80 g of initial weight) in a 4℃ room. Loin samples were then put in thin polyethylene bags and placed in a continuously heated water bath (80℃) until the core temperature, measured by a thermometer (Testo 108, Testo Inc., Germany), reached 71℃. Beef samples were then cooled in iced-water for 15 min. The cooled samples were then reweighed. Cooking loss was calculated as the percentage of weight loss after cooking (Honikel, 1998). Preparation of beef samples for WBS and TPA was similar to that for cooking loss measurement. At least 10 replicate cores (1.27 cm diameters) were used for the WBS analysis. WBS was determined using an Intron Universal Testing Machine (Model 1011, Instron Corp., USA) equipped with a Warner–Bratzler shearing device. TPA was performed using a TMS-Touch texture analyzer (Food Technology Corporation, USA). Eight meat pieces of 2×2×2 $cm^3$ (height×width×length) parallel to the muscle fiber direction were obtained for TPA analysis. TPA was measured by compressing to 80% with a compression probe, which moved downward at a speed of 3.0 mm/s (pre-test), 1.0 mm/s test speed, and a speed of 3.0 mm/s post-test. Textural properties of cooked beef, including hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness, were determined as described by Bourne (1978).

### Statistical analysis

To compare changes in marbling fleck traits within the LT muscle, muscle sample was classified into groups of low (<0.18; n=9) or high (≥0.18; n=5) CI based on the CI measured at the Korean carcass grading site. The general linear model (GLM) procedure was performed to elucidate associations among the groups and locations within the LT muscle using SAS software (2014). Statistical differences among the groups were detected by the probability difference (PDIFF), which was set at 5%. Results for the groups are presented as least squares means with standard errors.

## Results

### Comparison of carcass and marbling fleck characteristics

Coarseness of marbling fleck and carcass characteristics in the groups categorized by the degree of coarseness at the carcass grading site are shown in Table 1. As expected, the high CI group exhibited a greater area of large marbling fleck (coarseness) compared to the low CI group (0.23 vs. 0.14, $p$<0.001), even though no significant difference was observed in

**Table 1. Comparison of coarseness and carcass characteristics at the carcass grading site in the groups categorized by coarseness of marbling fleck**

| | Coarseness group | | Level of significance |
|---|---|---|---|
| | Low | High | |
| Coarseness | $0.14^b$ (0.01)[1] | $0.23^a$ (0.01) | *** |
| Marbling score | 7.80 (0.19) | 8.25 (0.30) | NS |
| Carcass weight (kg) | 453 (9.97) | 429 (15.8) | NS |
| Loin-eye area ($cm^2$) | 105 (1.83) | 103 (2.88) | NS |
| Back-fat thickness (cm) | 12.4 (1.79) | 11.3 (2.84) | NS |

Level of significance, NS, not significant; *** $p$<0.001.
[1] Standard error of least square means.
[a,b] Different superscripts in the same row represent significant differences ($p$<0.05).

marbling score between the high and low CI groups (8.25 vs. 7.80, $p$>0.05). Moreover, there were no significant differences in carcass weight, loin-eye area, and back-fat thickness between the marbling fleck groups ($p$>0.05).

The interaction between the coarseness of marbling fleck and longitudinal locations within LT muscle in the IMF content and marbling fleck characteristics is presented in Table 2. No significant differences were observed in the IMF content and marbling score among the groups ($p$>0.05). Location effects were detected in marbling and loin-eye areas, and these areas decreased from the central to front thoracic regions in the low or high CI groups ($p$<0.001). Due to a decrease in both the marbling and loin-eye areas, the percentage of marbling area did not differ among the regions in the low or high CI group ($p$>0.05), whereas the high CI group exhibited a higher percentage of marbling area compared to the low CI group (31.4 vs. 28.9%, $p$<0.05). A greater number of marbling fleck was observed in the low CI group compared to the high CI group (4,660 vs. 3,345, $p$<0.05), even though no significant difference was detected in the fineness index among the groups ($p$>0.05). The central region of low CI group had a lower coarseness compared to that of the high CI group (0.14 vs. 0.23, $p$<0.05). Marked differences were apparent in the F/C ratio between the low and high CI groups (32.9 vs. 17.6, $p$<0.05).

## Comparison of cooking loss and objective texture parameters

Least square means corresponding to cooking loss and objective tenderness parameters are presented in Table 3. No significant difference was observed in cooking loss among the CI and location groups ($p$>0.05). In the WBS, the central

**Table 2. Comparison of intramuscular fat (IMF) content and marbling characteristics in the groups categorized by coarseness of marbling fleck at the carcass grading site and longitudinal location of the *longissimus thoracis* muscle**

| Coarseness (C) | Low | | | High | | | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| Location (L) | Central (13/12T)[1] | Thoracic (9/8T) | Front thoracic (6/5T) | Central (13/12T) | Thoracic (9/8T) | Front thoracic (6/5T) | C | L | C×L |
| IMF content (%) | 20.9 (0.89)[2] | 19.4 (0.89) | 19.5 (0.89) | 21.9 (1.62) | 20.9 (1.62) | 20.6 (1.62) | NS | NS | NS |
| Marbling characteristics | | | | | | | | | |
| Marbling score | 7.80 (0.19) | 7.80 (0.19) | 7.90 (0.19) | 8.25 (0.31) | 8.25 (0.31) | 8.00 (0.31) | NS | NS | NS |
| Marbling area (cm$^2$) | 29.6$^a$ (1.20) | 27.0$^b$ (1.20) | 23.2$^c$ (1.20) | 32.6$^a$ (2.18) | 25.4$^{bc}$ (2.18) | 24.0$^{bc}$ (2.18) | NS | *** | NS |
| Loin-eye area (cm$^2$) | 105$^a$ (2.43) | 101$^a$ (2.43) | 86.9$^b$ (2.43) | 101$^a$ (3.83) | 88.8$^b$ (3.83) | 87.8$^b$ (3.83) | NS | *** | NS |
| Marbling area percentage (%) | 30.2$^{ab}$ (1.12) | 28.3$^b$ (1.12) | 28.4$^b$ (1.12) | 34.0$^a$ (2.04) | 30.3$^{ab}$ (2.04) | 29.8$^{ab}$ (2.04) | * | NS | NS |
| Number of marbling fleck | 4177$^a$ (430) | 4,868$^a$ (430) | 4,933$^a$ (430) | 3,230$^b$ (784) | 3,297$^b$ (784) | 3,509$^{ab}$ (784) | ** | NS | NS |
| Fineness (F) | 3.50 (0.10) | 3.43 (0.10) | 3.53 (0.10) | 3.16 (0.16) | 3.40 (0.16) | 3.41 (0.16) | NS | NS | NS |
| Coarseness (C) | 0.14$^{cd}$ (0.01) | 0.11$^d$ (0.01) | 0.10$^d$ (0.01) | 0.23$^a$ (0.02) | 0.19$^{ab}$ (0.02) | 0.17$^{bc}$ (0.02) | *** | *** | NS |
| F/C ratio | 26.5$^b$ (3.71) | 32.1$^{ab}$ (3.71) | 40.2$^a$ (3.71) | 13.7$^c$ (6.77) | 18.5$^c$ (6.77) | 20.8$^{bc}$ (6.77) | *** | NS | NS |

Level of significance, NS, not significant; * $p$<0.05; ** $p$<0.01; *** $p$<0.001.
[1] T, thoracic vertebrae.
[2] Standard error of least square means.
[a-d] Different superscripts in the same row represent significant differences ($p$<0.05).

**Table 3. Comparison of cooking loss and objective texture properties in the groups categorized by coarseness of marbling fleck at the carcass grading site and longitudinal location of the *longissimus thoracis* muscle**

| Coarseness (C) | Low | | | High | | | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|
| Location (L) | Central (13/12T)[1] | Thoracic (9/8T) | Front thoracic (6/5T) | Central (13/12T) | Thoracic (9/8T) | Front thoracic (6/5T) | C | L | C×L |
| Cooking loss (%) | 19.9 (0.84)[2] | 21.0 (0.84) | 23.7 (0.84) | 19.6 (1.38) | 21.3 (1.38) | 22.0 (1.38) | NS | NS | NS |
| WBS (N) | 54.2[a] (3.04) | 44.1[b] (3.04) | 48.1[ab] (3.04) | 39.5[b] (5.56) | 40.8[b] (5.56) | 38.8[b] (5.56) | * | NS | NS |
| Texture profile analysis | | | | | | | | | |
| Hardness (N) | 20.7 (1.03) | 18.0 (1.03) | 19.1 (1.03) | 19.5 (1.63) | 19.5 (1.63) | 17.8 (1.63) | NS | NS | NS |
| Adhesiveness (N · mm) | 0.85 (0.13) | 0.47 (0.13) | 0.65 (0.13) | 0.95 (0.21) | 0.34 (0.21) | 0.78 (0.21) | NS | NS | NS |
| Cohesiveness | 0.31 (0.01) | 0.28 (0.01) | 0.28 (0.01) | 0.28 (0.02) | 0.25 (0.02) | 0.29 (0.02) | NS | NS | NS |
| Springiness (mm) | 5.25 (0.21) | 5.38 (0.21) | 4.86 (0.21) | 5.03 (0.31) | 5.40 (0.31) | 4.86 (0.31) | NS | NS | NS |
| Gumminess (N) | 7.72[a] (0.43) | 5.26[b] (0.43) | 5.36[b] (0.43) | 7.51[a] (0.65) | 5.57[b] (0.65) | 5.11[b] (0.65) | NS | *** | NS |
| Chewiness (N · mm) | 45.2[a] (2.81) | 30.6[c] (2.81) | 30.1[c] (2.81) | 41.8[ab] (4.22) | 34.1[b] (4.22) | 26.0[c] (4.22) | NS | ** | NS |

Level of significance, NS, not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
[1] T, thoracic vertebrae.
[2] Standard error of least square means.
[a-c] Different superscripts in the same row represent significant differences ($p<0.05$).

region of the low CI group showed a higher value compared to the thoracic region of the low CI group (54.2 vs. 44.1 N, $p<0.05$), whereas the central region of the high CI group did not differ from the thoracic and front thoracic regions of the high CI group (39.5 vs. 40.8 and 38.8 N, $p>0.05$). Unlike the WBS value, there was no significant difference in TPA-hardness between the low and high CI groups ($p>0.05$). The central region of the high CI group was similar in hardness compared to the central and front thoracic regions of the low CI group (19.5 vs. 20.7 and 19.1 N, $p>0.05$). Like TPA-hardness, no significant differences were observed in adhesiveness, cohesiveness, and springiness among the groups categorized by coarseness of marbling fleck and location within the LT muscle ($p>0.05$). However, the central region of the low CI group had higher gumminess (7.72 vs. 5.36 N, $p<0.05$) and chewiness (45.2 vs. 30.1 N · mm, $p<0.05$) compared to the front thoracic region of the low CI group, whereas there were no significant differences between the low and high CI groups at the same location ($p>0.05$) with the exception of chewiness between the low and high CI groups in the thoracic region (30.6 vs. 34.1 N · mm, $p<0.05$).

## Discussion

Marbling scoring is an essential part of beef quality grading in the US, Japan, and Korea (Hocquette et al., 2010; Lee et al., 2018). In the meat industry, extensive efforts have been made to develop new methods to improve the accuracy and objectivity of the marbling assessment (Albrecht et al., 2006; Gerrard et al., 1996; Kuchida et al., 2000). Computer image analysis can be used to predict the marbling score and estimate marbling fleck traits (Albrecht et al., 2006). As image analysis technology significantly improves, as does the accuracy of analysis of the marbling fleck traits. Important marbling traits include the spatial distribution of marbling fleck, number of marbling fleck, and degree of coarser (coarseness) or smaller (fineness) marbling flecks within the *longissimus* muscle (Gerrard et al., 1996; Konarska et al., 2017). Kuchida et al. (2000) reported that the percentage of marbling area measured by computer image analysis was strongly correlated with the IMF content in the *longissimus* muscle of Japanese Black steer. An effect of breed on marbling area ratio was detected; Angus beef cattle had a greater area of marbling fleck in the *longissimus* muscle than did Belgian Blue cattle (Albrecht et al., 2006). Moreover, a higher IMF content of Japanese Black steer than that in European beef breeds was associated with a higher coarseness of marbling fleck (Peña et al., 2013). As the marbling score increases, bigger marbling flecks also increase in the bovine *longissimus* muscle (Konarska et al., 2017). Thus, marbling fleck characteristics varied in the LT muscle, and high-marbled beef loin had a higher coarseness compared to low-marbled beef loin. In this study, a marked difference was observed in the coarseness and F/C ratio between the groups categorized by the CI ($p<0.05$), even though there were no significant differences in the IMF content and marbling score between the low and high CI groups ($p>0.05$).

Within the *longissimus* muscle, the lumbar region ($5^{th}$ lumbar vertebrae) showed a higher IMF content, and the marbling area ratio and coarseness of marbling fleck were also greater in the lumbar region than in the central ($12^{th}$ thoracic vertebrae) or thoracic ($9^{th}$ thoracic vertebrae) regions (Nakahashi et al., 2007; Zembayashi and Lunt, 1995). In the porcine *longissimus* muscle, the marbling score of the central region (13T) did not differ considerably from those of the front thoracic (5T) or thoracic (9T) regions (Faucitano et al., 2004). Similar to the porcine *longissimus* muscle, in this study, no significant difference was observed in the marbling score and IMF content among the different locations, and total number and fineness of marbling fleck were also similar within the LT muscle ($p>0.05$). However, the location effect on the CI of marbling fleck was a greater in the LT muscle of Hanwoo steer, where the coarseness decreased from central to front thoracic regions in the low or high CI groups ($p<0.05$).

Estimations of location effects on tenderness have commonly been based on three to six locations within the muscle (Rhee et al., 2004; Williams et al., 1996). In the present study, beef loins from three different locations within the LT muscle were used. Contrastingly, locational variation in the WBS value may be associated with differences in the contraction speed and metabolic capacity within the muscle (Choi and Kim, 2009; Choi and Oh, 2016). Janz et al. (2006) reported that the WBS value was a greater in the central region (13T to 10T) than in the thoracic region (9T to 5T) within the LT muscle. Meanwhile, in this study, no significant location effects were observed in the WBS and TPA-hardness values among the three locations within the Hanwoo LT muscle ($p>0.05$), even though gumminess and chewiness decreased from the central to front thoracic region ($p<0.05$). A similar result was reported by Rhee et al. (2004), who suggested that there was no significant difference in the WBS value among the locations (posterior end, center, and anterior end) within the *longissimus* muscle. Additionally, Williams et al. (1996) reported a lower variation of the WBS value in the thoracic region (loin cut) compared to the lumbar region (sirloin cut) within the bovine *longissimus* muscle. Conversely, the high CI group exhibited a lower WBS value than did the low CI group (39.5 vs. 48.8 N, $p<0.05$). This result could be explained by a theory reported by Miller (2014), who suggested that as marbling flecks are less dense compared to lean tissue, high-marbled beef loin exhibited a lower toughness than low-marbled beef loin.

## Conclusion

Our results indicate that marbling fleck characteristics are variable within the LT muscle. In particular, marbling was coarser from the front thoracic to central regions ($p<0.05$), whereas no change in marbling fleck number was observed among the locations in the low or high CI groups ($p>0.05$). The effect of location on objective tenderness parameters within the Hanwoo LT muscle was somewhat limited, and muscles harboring a higher percentage of coarser marbling fleck showed a more tender beef compared to the muscles harboring a lower percentage of coarser marbling fleck ($p<0.05$).

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