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9 **Sensory quality and histochemical characteristics of *longissimus thoracis***
10 **muscle between Hanwoo and Holstein steers of different quality grades**

12 **Abstract**

13 This study compared the meat quality characteristics, palatability, and histochemical
14 characteristics of low-marbled Hanwoo and Holstein steers of different beef quality grades (1,
15 2, and 3). No differences were observed in muscle pH_{24 h} and cooking loss between the groups
16 (p>0.05); however, quality grade 1 of Hanwoo steers (HA1) showed a darker muscle surface
17 compared to grade 1 of Holstein steers (HO1) (30.9 vs. 33.9, p<0.05). The HA2 group
18 exhibited a lower value of Warner-Bratzler shear force compared to the HO1 and HO3 groups
19 (60.8 vs. 69.2 and 87.8 N, p<0.001). For sensory quality attributes, steaks from the HA1
20 group showed higher scores of softness, initial tenderness, and amount of perceptible residue
21 than steaks from the HO1 group (p<0.001). Within the quality grade 2, Hanwoo steers had a
22 higher score of softness compared to Holstein steers (p<0.001). There were no differences in
23 juiciness and flavor intensity between Hanwoo and Holstein steers at the same quality grade
24 (p>0.05). This difference in tenderness attributes between the breeds within the quality grade
25 was associated with morphological traits of muscle bundle, and Hanwoo steers had smaller
26 bundle area (0.37 vs. 0.50 mm², p<0.05) and higher fiber number per bundle (88.2 vs. 121,
27 p<0.05) compared to Holstein steers. Therefore, bundle characteristics of *longissimus thoracis*
28 muscle can be crucial for explaining factor for the explanation of tenderness variations
29 between different breeds at the same beef quality grade or marbling.

30
31 **Key words:** Sensory quality, Muscle bundle, Hanwoo, Holstein, Beef quality grade

33 **Introduction**

34 It is well known that, among the sensory quality characteristics of cooked beef,
35 tenderness is a critical determinant of overall palatability (Hulankova et al., 2018; Miller et al.,
36 2001), and it largely contributes to the satisfaction, dissatisfaction, and purchase-related
37 decision of consumers (Choi and Kim, 2009; Lee et al., 2019). In response, the meat industry
38 has been making efforts to improve tenderness and produces beef with a consistent tenderness
39 (Anderson et al., 2012). In contrast, beef palatability is primarily the interactive result of
40 various genetic and environmental factors, such as breed, gender, age, muscle type, and
41 feeding regime (Hulankova et al., 2018; Koohmaraie et al., 2002; Lee et al., 2018). Among
42 these factors, cattle breed is an important determinant to cause variations in tenderness, as
43 significant differences were observed in the intramuscular fat (IMF) contents and
44 histochemical characteristics of skeletal muscles from the different breeds (Albrecht et al.,
45 2006). In general, Korean consumers tend to prefer beef with higher beef marbling standard
46 (BMS) scores, since high-marbled beef exhibited greater acceptability of tenderness, juiciness,
47 and flavor compared to those of low-marbled beef (Lee and Choi, 2019). Primarily, the
48 Hanwoo breed has the potential to deposit a higher degree of marbling compared to the other
49 cattle breeds, such as the Holstein breed (Lee et al., 2019), and exhibited a greater appearance
50 rate of 1⁺⁺ and 1⁺ grades than the other meat-type breeds (48.0% vs. 3.3%) according to the
51 carcass grading standard of Korean Institute of Animal Products Quality Evaluation (KAPE,
52 2021).

53 Within the quality grade or BMS score, differences in the palatability can exist between
54 the Hanwoo and the other meat-type breeds due to the other factors influencing tenderness
55 (Lee et al., 2018). The morphological and metabolic characteristics of skeletal muscle can
56 also be an essential factor in determining the organoleptic characteristics of cooked beef (Choi
57 and Kim, 2009). Choi et al. (2019) reported that muscle bundle area was negatively correlated

58 with the sensory tenderness attributes conducted by the trained panelists. Moreover, Angus,
59 known as a typical beef breed, had a smaller bundle area (Albrecht et al., 2006), and exhibited
60 a lower Warner-Bratzler shear force (WBS) compared to double-muscled Belgian blue,
61 known as an extreme breed for muscle growth (Wheeler et al., 2001). Thus, muscle bundle
62 characteristics are associated with the variations in sensory tenderness, as muscle bundle, a
63 group of muscle fibers, is responsible for maintaining the surface stiffness in living and
64 postmortem muscles (Lee et al., 2018; Schleip et al., 2006).

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

73 **Materials and Methods**

74 **Animals and muscle samples**

75 Fifty-nine steers (35 Hanwoo [quality grade 1, n = 23; quality grade 2, n = 12] and 24
76 Holstein [quality grade 1, n = 8; quality grade 2, n = 10; quality grade 3, n = 6]) were obtained
77 in six batches (11–12 Hanwoo steers per day and 8 Holstein steers per day). Approximately
78 25 g of muscle samples was taken for the histochemical analysis from the LT muscle at the
79 12–13th thoracic vertebrae, and immediately frozen in liquid nitrogen, and stored at –80°C.

80 At 24 h postmortem, the carcasses from Hanwoo and Holstein steers were graded
81 following the carcass quality standards of the KAPE (2021). The KAPE provided the age at
82 slaughter, carcass weight, loin-eye area, back-fat thickness, marbling scores, and beef quality

83 grades of each carcass. However, quality grade 3 of Hanwoo steers was excluded from all the
84 statistical analyses because data were obtained from only two steers. After quality grading,
85 muscle chunks were dissected between the 9–13th thoracic vertebrae, and the meat quality
86 measurements were immediately conducted. For the eating quality evaluation, each muscle
87 chunk was cut into steak-sizes (1.5 cm thickness; approximately 120 g), and then stored at –
88 25°C.

89

90 **Beef quality measurements**

91 Muscle pH was assessed after carcass quality grading using a portable pH meter (Testo
92 206-pH2, Testo AG, Lenzkirch, Germany). After 30 min of blooming at 4°C in a cold room,
93 the meat surface color was measured using a chromameter (CR-400, Minolta Camera Co.,
94 Osaka, Japan), and values were expressed as lightness (L*), redness (a*), and yellowness (b*)
95 (Commission Internationale de l’Eclairage, 1978). To measure the water-holding capacity,
96 drip loss and cooking loss were measured according to the previously reported procedure
97 described by Honikel (1998). After cooking loss measurement, the same samples were used
98 for the WBS analysis. Each meat sample was cut into more than 8 cores (1.27 cm diameter),
99 and the force values were measured using an Instron Universal Testing Machine (Model 1011,
100 Instron Corp., Canton, USA) mounted with a Warner-Bratzler blade operating at a crosshead
101 speed of 200 mm/min (American Meat Science Association; AMSA, 1995).

102

103 **Sensory quality evaluation**

104 Sensory quality analysis was conducted using 59 meat samples from Hanwoo and
105 Holstein steers during 12 sessions (4–5 beef loins per session). Ten trained panels (five
106 females and five males; ages 24–46 years) were used in this study. Approval was granted by
107 the Kyungpook National University (KNU) Bioethics Committee (protocol number 2019-

108 0027). Sensory panel training was performed for at least 6 mon at the Muscle Biology
109 Laboratory of KNU according to the guidelines of AMSA (1995). Sensory evaluation was
110 performed based on previously reported procedure (Lee and Choi, 2019; Lee et al., 2019).
111 The frozen samples were thawed at 4°C for 18 h. Beef steaks were cooked by pan-frying with
112 turn-over every 3min, until the core temperature reached 71°C using an induction range (CIR-
113 IH300RGL, Cuchen, Cheonan, Korea). Trained panelists evaluated the sensory quality
114 characteristics of cooked beef loins, including five tenderness attributes, juiciness, flavor
115 intensity, off-flavor intensity, and mouth coating, using a 9-point scale.

116

117 **Histochemical analysis**

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

125

126 **Statistical analysis**

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

132

133 **Results**

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

136 Table 1 shows the results of meat quality characteristics of the bovine LT muscle in each
137 group. As expected, the marbling score was clearly different depending on the quality grades
138 regardless of breeds ($p < 0.001$). No significant difference was detected in muscle pH_{24h}
139 among the groups ($p > 0.05$). For the meat color, Holstein steers exhibited a higher lightness
140 value compared to Hanwoo steers ($p < 0.05$), although there was no difference between the
141 HA1 and HO2 groups (30.9 vs. 32.7, $p > 0.05$). Redness and yellowness values did not
142 significantly differ among the groups ($p > 0.05$). Although samples from the HA2 group
143 exhibited a higher drip loss compared to samples from the other groups ($p < 0.05$), cooking loss
144 was not different among all groups ($p > 0.05$). There was no difference in WBS value within
145 the Hanwoo group ($p > 0.05$); whereas a marked difference was observed between the quality
146 grades in Holstein steers ($p < 0.001$).

147

148 **Comparison of sensory quality characteristics between Hanwoo and Holstein steers**

149 Fig. 1 displays sensory quality characteristics of cooked beef from each group evaluated
150 by trained panelists. From all tenderness attributes, statistically significant differences were
151 found among the groups ($p < 0.001$). Softness and initial tenderness scores decreased as the
152 quality grades decreased within Hanwoo or Holstein steers, and softness score tended to be
153 lower in the Holstein group than in the Hanwoo group within the same grade ($p < 0.001$).
154 However, quality grade 2 samples of Hanwoo and Holstein steers showed a similar score in
155 initial tenderness (5.10 vs. 5.06, $p > 0.05$). There were no differences in chewiness and rate of
156 breakdown between the Hanwoo and Holstein steaks within the same grade ($p > 0.05$),
157 although significant differences were observed among the quality grades within the breed

158 ($p < 0.001$). No difference was observed in the amount of perceptible residue between the HO2
159 and HA2 groups (4.86 vs. 5.22, $p > 0.05$). In Holstein steers, a significant difference was
160 detected in juiciness score among the grades, and the HO1 group exhibited a higher juiciness
161 compared to the other grades ($p < 0.001$). The HA2 group had a similar flavor intensity
162 compared to the other groups ($p > 0.05$) except the HO1 group (5.75 vs. 6.25, $p < 0.01$), and
163 there was no difference in off-flavor intensity among the groups ($p > 0.05$). Mouth coating
164 score significantly differed between the quality grades within Hanwoo or Holstein breeds
165 ($p < 0.001$).

166

167 **Comparison of histochemical characteristics between Hanwoo and Holstein steers**

168 The histochemical characteristics of Hanwoo and Holstein steers from each quality grade
169 are shown in Fig. 2. No significant difference was observed in muscle fiber area among the
170 groups ($p > 0.05$). Due to smaller loin-eye area and similar fiber size, the Holstein steers
171 exhibited a fewer total fiber number compared to the Hanwoo steers ($p < 0.01$). However, the
172 HA2 group had a similar number compared to the Holstein group ($p > 0.05$). For the muscle
173 bundle characteristics, Holstein steers showed a greater bundle area compared to the Hanwoo
174 steers ($p < 0.001$). Higher fiber number per bundle was observed in the HO3 group compared
175 to the HA1 and HA2 groups (138 vs. 89.9 and 83.7, $p < 0.001$). In contrast to the bundle area,
176 the total bundle number was lower in the Holstein group compared to the Hanwoo group
177 ($p < 0.001$).

178

179 **Discussion**

180 Beef quality grade is an objective evaluation for sorting a heterogeneous population into
181 homogeneous groups based on their organoleptic beef characteristics. A crucial factor in
182 determining the quality grade is the degree of marbling at the standard site (LT muscle surface

183 at the 13th thoracic vertebra) for carcass grading (Lee et al., 2019). Generally, the Hanwoo
184 breed applies a long-term fattening regime to achieve a higher marbling score; thus, it was
185 reported to have greater quality grades compared to those of the other breeds in Korea (Lee et
186 al., 2019; KAPE, 2021). In contrast, Holstein cattle have a significantly lower IMF content in
187 the LT muscle compared to beef breeds, since dairy breeds are typically selected based on
188 their milk production ability rather than muscle mass and meat quality (Rezagholidvand et al.,
189 2021). At the same live weight, Holstein cattle have a lower ratio of carcass weight and poor
190 muscularity compared to beef breeds due to a higher proportion of non-carcass parts,
191 including liver, heart, and kidney (Bown et al., 2016). Moreover, these steers often exhibited a
192 thinner back-fat compared to Angus steers, although the marbling score was similar between
193 the two breeds at the same live weight and at the same maturity level (Muir et al., 2000).
194 Compared with Hanwoo and Holstein steers in this study, carcasses derived from Hanwoo
195 steers had greater loin-eye area and back-fat thickness compared to carcasses derived from
196 Holstein steers at approximately similar carcass weight ($p < 0.001$, Supplementary Table S1).
197 For meat quality traits, Hanwoo steers exhibited a darker muscle surface compared to
198 Holstein steers ($p < 0.05$), and no difference was observed in cooking loss between carcasses
199 from the two breeds ($p > 0.05$). Bown et al. (2016) compared the meat quality traits between
200 Holstein, Hereford, and Hereford×Holstein steers at the same age, and who reported no breed
201 difference in WBS level. Furthermore, no difference was reported in WBS level among
202 Holstein select, Holstein choice, and Charolais cross-bred choice following the United States
203 Department of Agriculture grades (Schaefer et al., 1986). Interestingly, in this study, at the
204 same quality grade, steaks from Hanwoo steers showed a lower WBS level compared to
205 steaks from Holstein steers ($p < 0.001$). Thus, breed differences were somewhat existed in the
206 carcass and meat quality characteristics between Hanwoo and Holstein steers in this study.

207 In tenderness attributes, within the quality grade 1, the Hanwoo group exhibited greater

208 scores of softness, initial tenderness, and amount of perceptible residue compared to the
209 Holstein group ($p < 0.001$). A higher softness score was also observed in Hanwoo steers
210 compared to Holstein steers at the quality grade 2 ($p < 0.001$), although the other tenderness
211 attributes were not different between the two breeds ($p > 0.05$). Armbruster et al. (1983)
212 suggested that loin steaks from Angus steers (well-known as a beef breed) required lower
213 force for penetration and fewer chews before swallowing compared to loin steaks from
214 Holstein steers. However, no cattle breed effect was observed by taste panel in tenderness
215 between Holstein and the other breeds, including Hereford, Charolais, and Jersey (Bown et al.,
216 2016; Schaefer et al., 1986). In contrast, there were generally no differences in juiciness,
217 flavor, and off-flavor between beef and dairy breeds when compared at the same marbling
218 degree (Bown et al., 2016; Schaefer et al., 1986). These results support the findings of the
219 present study. There were no significant differences in juiciness and flavor intensity between
220 two breeds within the same quality grade ($p > 0.05$). Altogether, steaks from typical beef breeds
221 could be tender compared to steaks from dairy breeds, although no breed effects could be
222 observed in the other sensory quality traits at the same quality grade or marbling degree.

223 Muscle bundle and perimysium play an essential role in load and stress bearing functions
224 under various conditions, and are therefore associated with the integrity of contraction and
225 relaxation of living muscles (Gillies and Lieber, 2011; Schleip et al., 2006). It is well known
226 that the bundle characteristics, especially bundle size and fiber number per bundle, are the
227 cause of variations in the texture feature and firmness of muscle surface during the
228 postmortem periods (Lee et al., 2018). On the other hand, a clear difference was observed in
229 fiber number per bundle or bundle size between the cattle breeds (Albrecht et al., 2013;
230 Norman, 1982). Norman (1982) reported that the Charolais breed harboring a smaller bundle
231 area showed a lower WBS value compared to Nelore breed harboring a greater bundle area.
232 Within the quality grade, Hanwoo steers harboring a greater fiber number per bundle

233 exhibited visually coarser texture and less tender meat than Hanwoo steers harboring a lower
234 number per bundle (Lee et al., 2018). These findings on the effects of bundle characteristics
235 agree well with the results of this study. Steaks from Hanwoo steers showing a smaller bundle
236 area exhibited a lower required force for the initial few chewing than steaks from Holstein
237 steers showing a larger bundle area within the quality grade ($p<0.05$).

238

239 **Conclusion**

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

243

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311

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312 **Figure caption**

313

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

321

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

328

329 **Table 1. Comparison of marbling score and meat quality characteristics between Hanwoo and Holstein steers of different beef quality**
 330 **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) | 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) ¹ | 5.52 (0.04) | 5.62 (0.05) | 5.58 (0.04) | 5.61 (0.06) | NS |
| <i>Meat color</i> | | | | | | |
| Lightness (<i>L</i> [*]) | 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 (<i>a</i> [*]) | 16.4 (0.48) | 16.4 (0.67) | 17.9 (0.82) | 17.5 (0.73) | 18.9 (0.94) | NS |
| Yellowness (<i>b</i> [*]) | 8.32 (0.49) | 7.55 (0.68) | 8.46 (0.84) | 7.94 (0.75) | 8.66 (0.97) | NS |
| <i>Water holding capacity</i> | | | | | | |
| 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 |

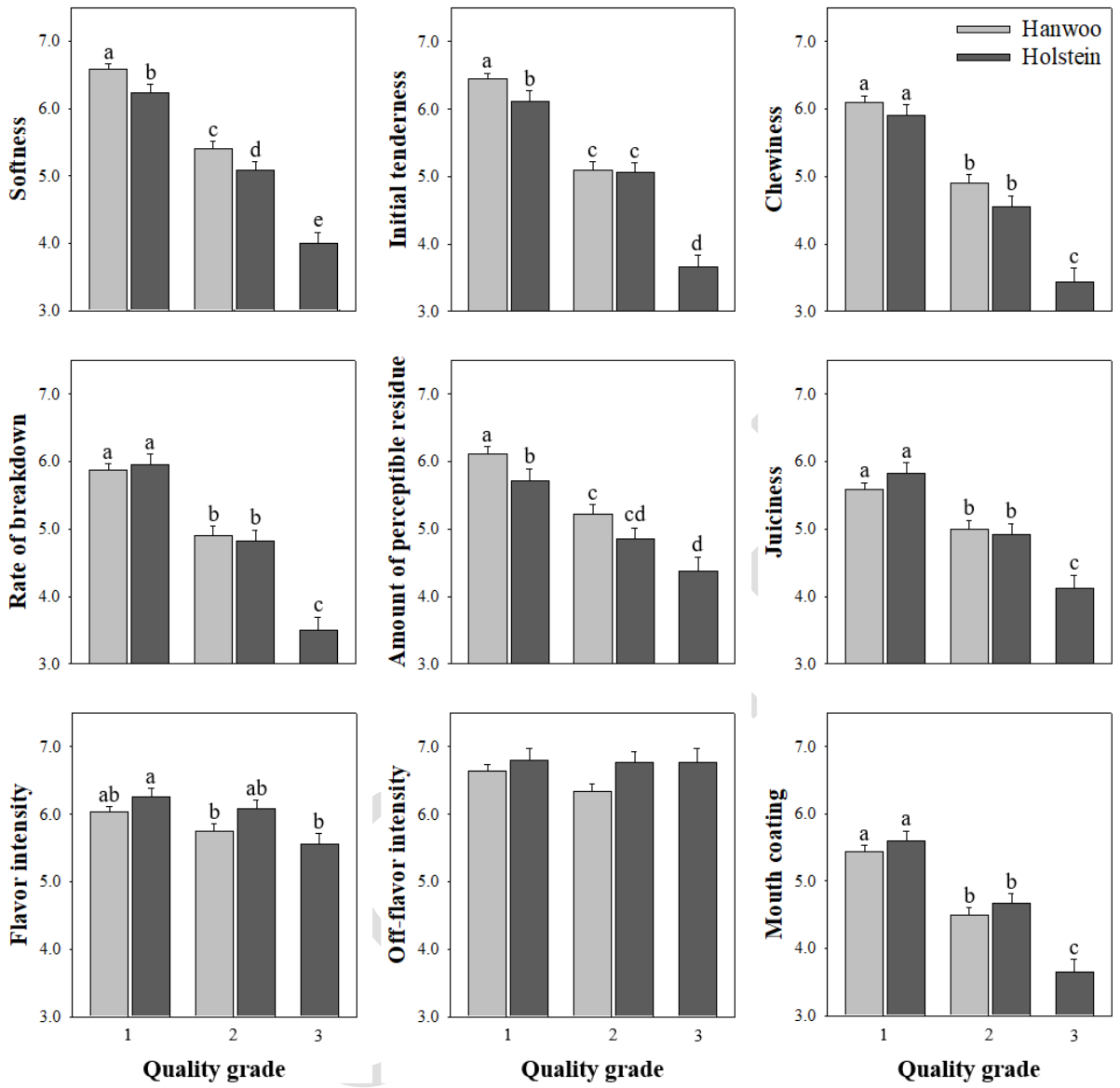
| | | | | | | |
|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----|
| Warner-Bratzler shear force (N) | 56.6 ^d (1.68) | 60.8 ^d (2.43) | 69.2 ^c (2.90) | 78.7 ^b (2.43) | 87.8 ^a (3.44) | *** |
|---------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----|

331 Level of significance: NS, not significant; * p<0.05; *** p<0.001.

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

333 ¹ Standard error of least square means.

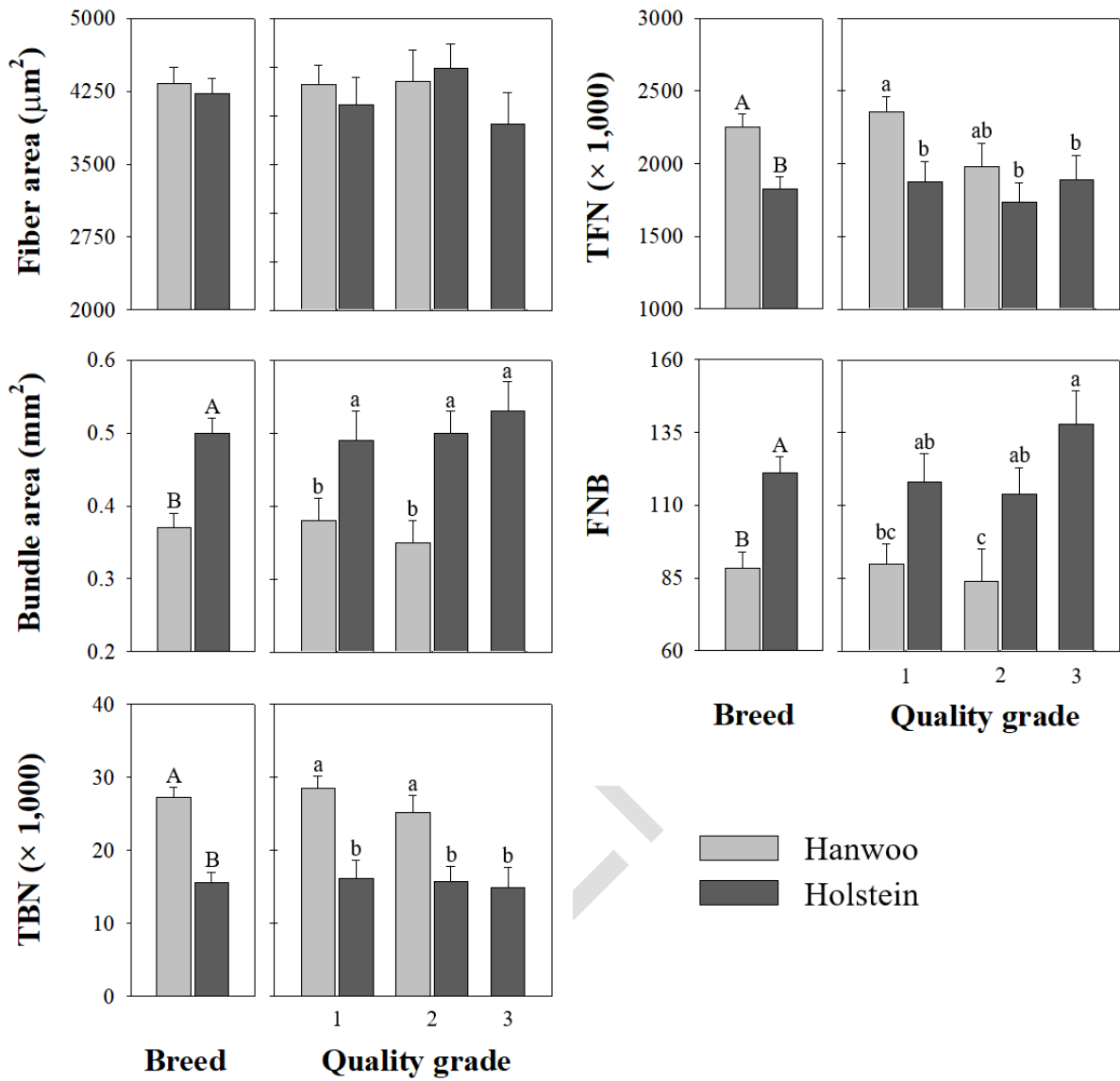
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337 **Fig. 2.**



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Supplementary Table S1. Comparison of carcass characteristics between Hanwoo and Holstein steers of different beef quality grades

| Breed Quality grade | Hanwoo | | Holstein | | | Level of significance |
|----------------------------------|--|-----------------------------|-----------------------------|-----------------------------|------------------------------|--------------------------|
| | 1 | 2 | 1 | 2 | 3 | |
| Age at slaughter (mon) | 31.2 ^a (0.38) ¹ | 30.0 ^a (0.63) | 23.9 ^b (0.45) | 22.3 ^c (0.40) | 22.5 ^{bc} (0.52) | *** |
| Carcass weight (kg) | 467 (11.2) | 422 (11.5) | 442 (19.0) | 435 (17.0) | 419 (22.0) | NS |
| Loin-eye area (cm ²) | 98.9 ^a (1.91) | 90.6 ^b (2.65) | 77.5 ^c (3.24) | 75.6 ^c (2.90) | 73.7 ^c (3.75) | *** |
| Back-fat thickness (mm) | 14.2 ^a (0.83) | 13.8 ^a (1.14) | 9.88 ^b (1.40) | 8.90 ^b (1.25) | 8.83 ^b (1.62) | *** |

340 Level of significance: NS, not significant; *** p<0.001.

341 ^{a-c} Different superscripts in the same row represent significant differences (p<0.05).342 ¹ Standard error of least square means.

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