

TITLE PAGE

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9 **Effect of boiled feed on the physicochemical, histochemical, and nutritional properties**
10 **of Hanwoo cow beef (*M. longissimus lumborum*)**

11 **Abstract**

12 The aim of this study was to investigate the effect of boiled feed on meat quality as a
13 fundamental study to establish a standardized feeding regime for cows. Cow beef strip loins
14 (*M. longissimus lumborum*) from two different cow groups (n = 10 each; CON, finishing with
15 normal feed; TR, finishing with boiled feed) were used to evaluate muscle fiber
16 characteristics, meat quality traits, and fatty acid and amino acid composition. The cut surface
17 of the 7th-8th thoracic vertebrae was used to analyze muscle characteristics. Muscle and
18 muscle fiber characteristics were not significantly different between the CON and TR groups
19 ($P > 0.05$). Purge loss was lower in the TR group, and redness, yellowness, and cooking loss
20 after 2 weeks of aging were also lower in the TR group ($P < 0.05$). The TR group showed
21 significantly higher unsaturated fatty acid and lower saturated fatty acid content than the
22 CON group ($P < 0.05$). No significant differences were found in amino acid composition ($P >$
23 0.05). These results indicate that cow productivity can be improved by finishing with boiled
24 feed, thereby lowering production costs and improving carcass grade. Additionally, it
25 provides a better perception of consumer beef as healthier.

26 **Key words:** cow beef, boiled feed, meat quality, fatty acid composition

27 **1. Introduction**

28 In South Korea, it has been shown that the number of bovine slaughter heads
29 exceeded 1.0 million in 2023, and 1.1 million in 2024 (KAPE, 2024; KMTA, 2025). Among
30 Hanwoo heads, 451,000 were steers and 411,000 were cows in 2023, 455,000 were steers,
31 and 469,000 were cows in 2023. This is a well-established feeding standard for maximizing
32 Hanwoo steer productivity. However, in the case of cows, this is not applicable because most
33 are culled cows due to the loss of productivity, as their quality grades were poor due to
34 excessive subcutaneous fat and lack of marbling resulting from oversupplementation of
35 concentrate and a short finishing period (NIAS, 2022). In addition, there are several
36 limitations to establishing a feeding standard, such as an unspecific slaughter age. Thus, the
37 cow feeding regime is extremely dependent on the farmer. Considering that the quality grade
38 of cows is poorer than that of steers, and the number of heads is similar, it is necessary to
39 conduct additional research on cow feeding to increase farm productivity and consumer
40 satisfaction.

41 Due to the rise in international grain prices, the economic burden on concentrated
42 feed has increased, leading some farms to reduce beef production costs by using feed they
43 produce themselves, such as various agricultural by-products and forage (Choi et al., 2024).
44 In terms of reducing production cost, 'boiled feed' is a feeding regime that involves boiling
45 agricultural by-products in a large cauldron, and it originated from traditional agrarian society
46 (Kim et al., 2021). Generally, boiled feeding has an economic advantage because it utilizes
47 by-products such as rice straw and rice bran (Choi et al., 2017). In addition, a previous study
48 reported that boiling is beneficial to rumen digestibility, since the silica-lignin-cellulose
49 binding of forage, which is difficult to digest, is weakened by the boiling procedure (Zhang et
50 al., 2017). Choi et al. (2017) reported a better yield grade of boil-fed steers by decreasing

51 carcass weight and back fat thickness and increasing loin-eye area, whereas there were no
52 significant differences in quality grade. For this reason, some farms adopt underburden cost
53 boiled feeding to overcome low profitability owing to the comparatively lower quality grade
54 compared to steers.

55 Thus, the aim of this study was to investigate the quality grade and meat quality of
56 beef fed with common/boiled feed as a fundamental study in order to establish a standardized
57 feeding regime or cow feeding standard, including technology for improving cow beef
58 quality.

59

60 **2. Materials & methods**

61 **2.1. Sample preparation**

62 Beef cows which were finished with Korean Feeding Standard for Hanwoo (NIAS,
63 2022) (CON; n = 10) or boiled feed (TR; n = 10) were selected from a commercial
64 slaughterhouse. The details of the feed and feed nutrients for the CON and TR groups are
65 shown in Table 1. Age and carcass weight of beef cow selected were 42.7 ± 2.7 months and
66 416.3 ± 10.2 kg for CON and 42.0 ± 2.8 months and 400.4 ± 9.1 kg for TR, respectively. The
67 entire loin (from 1st thoracic vertebra to the 6th lumber vertebra) was removed from the left
68 side of the carcasses 24h postmortem. The cut surface images of loin obtained between 7th
69 and 8th thoracic vertebra were used to analyze loin-eye (*M. longissimus thoracis*) area (cm²),
70 *M. spialis thoracis* area (cm²), and intermuscular fat area (cm²) between *M. longissimus*
71 *thoracis* and *M. spialis thoracis*. Strip loin (*M. longissimus lumborum*) was cut into two
72 pieces (10 cm thickness), vacuum packed, and stored in a cold room at 4 °C. Strip loins were
73 removed from the packages at 48 h postmortem (day 2) and 15 days after storage (day 16)

74 and used to evaluate meat quality, muscle fiber characteristics, and fatty acid and amino acid
75 compositions.

76 **2.2. Intermuscular fat and loin-eye area**

77 The intermuscular fat area between the *M. longissimus thoracis* and *M. spinalis thoracis*, the
78 areas of the loin eye (*M. longissimus thoracis*) and *M. spinalis thoracis*, and the ratio of fat to
79 lean were analyzed using Image Pro Plus (Media Cybernetics, Rockville, MD, USA)
80 according to the method described by Im et al. (2024) with some modifications.

81 **2.3. Muscle fiber characteristics**

82 The muscle fiber characteristics of bovine striploins were evaluated using an
83 immunohistochemical staining method described by Song et al. (2020). Briefly, frozen
84 samples in methyl butane were sliced into 10 μm transversal section using a cryostat
85 microtome (CM1520; Leica Biosystems, Wetzlar Germany) at $-20\text{ }^{\circ}\text{C}$. Samples were blocked
86 immediately in 10% normal goat serum (Cell Signaling Technology, Danvers, MA, USA),
87 then, stained with primary antibodies specific to myosin heavy chain (MHC) isoforms (BA-
88 F8, SC-71, BF-35, and 6H1; DSHB, Iowa City, IA, USA). Fluorescent dye-conjugated
89 secondary anti-IgG and anti-IgM antibodies (Alexa Fluor 405, 488, and 594; Thermo Fisher
90 Scientific) were used for incubation. Stained muscle fibers were visualized under a
91 fluorescence microscope (EVOS M5000; Thermo Fisher Scientific, Waltham, MA, USA).
92 Three fields of each sections were used to analyze with Image Pro Plus Program (Media
93 Cybernetics, Rockville, MD, USA), and cross-sectional area (CSA; μm^2), relative fiber
94 area/number (%), and density (number/ mm^2) of each fiber type were analyzed.

95 **2.4. Proximate composition and meat quality properties**

96 The proximate composition of strip loin from cows was determined using the
97 method of AOAC (2000) for moisture and crude ash, the method of Kjeldahl (AOAC, 2000)

98 for crude protein, and Folch et al. (1957) for crude fat with modifications. 10 g of samples
99 were dried at 105 °C for 24 h using dry oven (DH.WOC00560, Daihan Scientific, Wonju,
100 Republic of Korea). Crude ash was analyzed by drying at 200 °C then, burned at 400 °C,
101 600 °C, and 800 °C for 2 h. Both moisture and crude ash were expressed as percentage by
102 calculate using before and after weight. Following the Kjeldahl method (AOAC, 2000),
103 samples (0.5 g) were digested with sulfuric acid and ammonia and then distilled into boric
104 acid. Crude protein content was obtained by multiplying the total nitrogen content by 6.25
105 using titrated borate anions with hydrochloric acid. For the crude fat contents, 5.0 g of each
106 sample was homogenized in 35 mL of Folch solution (chloroform:methanol, 2:1, v:v) and 1
107 mL of internal fatty standard (C13:0, 0.5 mg/mL in methanol) for further fatty acid analysis.
108 The homogenates were then filtered through Whatman No. 1 filter paper (Merck, Darmstadt,
109 Germany). By adding 0.88% NaCl, filtrate was separated into two layers. After the upper
110 layer was removed, 10 mL of the lower layer was collected and evaporated with nitrogen gas.
111 Crude fat content was expressed as a percentage of the sample.

112 For the meat color, D65 light source, 8° illumination, and 8 mm measuring aperture
113 equipped colorimeter (CR-400; Minolta Co., Tokyo, Japan) was utilized to instrumental color
114 measurement after 20 min of blooming at 4 °C. The colorimeter was calibrated using a white
115 plate ($Y = 93.5$, $x = 0.3132$, and $y = 0.3198$). The Commission Internationale de l'Éclairage
116 system (Commission Internationale de l'Éclairage, 1978) was adopted in order to express the
117 results in terms of lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*). Three
118 grams of samples were homogenized in 27 mL distilled water. The pH was determined
119 immediately using a pH meter (S220; Mettler Toledo, Greifensee, Switzerland) equipped with
120 a temperature-adaptation probe. Before the measurement, calibration was conducted using
121 standard buffer solutions with pH 4.01, 7.00, and 9.21 at 20 °C. Drip and cooking losses were
122 measured to evaluate water-holding capacity. Drip loss was measured by suspending 50 g of

123 sample for 24 h at 1 °C, and calculated using before and after suspension weight as described
124 by Honikel (1987). Cooking loss was measured as the change in weight before and after
125 cooking in a water bath (WB-22, Daihan Scientific, Wonju, Republic of Korea). Sixty
126 samples were randomly assigned to six batches of ten samples each. Cooking was
127 accomplished when the internal temperature reached to 70 °C, and cooled for 30 min at room
128 temperature, then samples were weighted. The results were expressed as a percentage of the
129 initial weight. From the cooked samples, three cores of 1 cm in diameter were obtained
130 following muscle fiber orientation. A texture analyzer (TA1; Ametek, Berwyn, PA, USA)
131 equipped with a Warner–Bratzler shear blade was used to determine the Warner-Bratzler
132 shear force (WBSF). The shear force values were recorded as N/cm² and were obtained by
133 vertical shearing at 3.00 mm/s speed with a 50 kgf of load cell capacity.

134 **2.5. Fatty acids and amino acids analysis**

135 Fatty acids were analyzed as described by Kim et al. (2021). Briefly, 1 mL of
136 dichloromethane was added to the evaporated sample to analyze the crude fat content, which
137 was then transferred to a glass tube and reacted with 1 mL of 1 N methanolic NaOH for
138 saponification. After heating in the heating block at 90 °C for 10 min and cooling at room
139 temperature for 20 min, 1 mL of 14% boron trifluoride-methanol solution was added for
140 subsequent methylation. After heating and cooling, 3 mL hexane and 8 mL distilled water
141 were added, and the mixture was allowed to stand overnight for layer separation. One
142 milliliter of upper layer was moved to vial and used for fatty acid analysis using a GC
143 machine (8890, Agilent Technologies Inc., CA, USA) equipping Supleco SP-2560 capillary
144 column (100m x 0.25 mm i.d., 0-20 µm film thickness; Sigma-Aldrich Co., MO, USA).
145 Detail conditions of the GC are as follows; inlet temperature, 220 °C; split mode with 10:1
146 split ratio; oven temperature, 100 °C (4 min) at 25 °C/min to 200 °C (8 min), and at 5 °C/min

147 to 250 °C (6 min); injection volume, 1 µL; column flow, 2.4 mL/min of nitrogen as carrier
148 gas; detector temperature, 250 °C. A fatty acid methyl ester (FAME) standard (FAME Mix
149 CRM-47885, Sigma-Aldrich Co., St Louis, MO, USA) was used to identify the samples and
150 compare their retention times. The total fatty acid content was expressed in milligrams per
151 gram of sample. The fatty acid composition was expressed as a percentage of total fatty acids
152 after quantification using the peak area of an internal standard.

153 For the amino acids analysis, 0.2 g of dried samples were hydrolyzed in 5 mL of 6 N
154 HCl at 110 °C for 24 h. Hydrolysate were filtered through Whatman No. 1 filter paper, and
155 adjust to 10 g filtrate with distilled water. After vortex, 1 mL of mixture filtered through a
156 0.2-µm membrane filter (Phenomenex, USA). The analyses were performed using a Dionex
157 Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA).
158 Chromatographic separation was achieved with a Inno C18 Column (150 × 4.6 mm, 5.0 µm;
159 Youngjinbiochrom, Sungnam, Republic of Korea). Gradient elution was performed using a
160 40 mM sodium phosphate buffer (solvent A; pH 7) and water/acetonitrile/methanol (solvent
161 B; 10:45:45, v/v/v). The following binary mobile phase linear gradients were used: 95% A at
162 0 min, 45% A at 24 min, 20% A at 25 min, and 95% A at 34.5 min. The column temperature
163 and flow rate were 40 °C and 1.5 mL/min, respectively. The detection was performed using a
164 fluorescence detector. Two derivatizing agents, o-phthaldialdehyde (OPA; Agilent
165 Technologies Inc., Santa Clara, CA, USA) and FMOC (9-fluorenylmethoxycarbonyl
166 chloride; Agilent Technologies Inc.), were used simultaneously according to the
167 manufacturer's instructions. The excitation/emission wavelengths were 340/450 nm for the
168 OPA-derivatized amino acids and 266/305 nm for the FMOC-derivatized amino acids. The
169 concentrations of individual amino acids were determined using five-point calibration curves
170 of an Amino Acid Standard (WAT088122, Waters Co., Milford, MA, USA).

171 **2.6. Statistical analysis**

172 All experimental data obtained from two different treatments (CON and TR) and two
173 storage times (Day 2 and Day 16) with technical triplicates were expressed as means and
174 standard error (SE). Student's t-test was performed to compare muscle fiber characteristics,
175 meat quality traits, fatty acids, and amino acids between the two groups of beef cows and two
176 storage days (SAS software, ver. 9.4, SAS Institute, Carry, USA). Differences were
177 considered statistically significant at $P < 0.05$.

178

179 **3. Results & discussion**

180 Fig. 1. shows the carcass grade, appearance rate, age, and carcass weight distribution of
181 slaughtered cow carcasses in 2023. Among the total cow carcasses, grade 1 showed the
182 highest appearance rate (27%), the same as a selected farm, while the ratio of grade 1 in the
183 selected farm was 44%. The appearance rate of 1++ grade was 2% higher in total, that of 1+
184 grade was 5% higher in selected farms. And the ratio of grades lower than 1 (grades 2 and 3)
185 was lower in selected farms (21%) than total (41%). Distribution of age of slaughtered cows
186 older than 66 months showed highest ratio (26%) in total, whereas distribution of 35 – 44
187 months' age was highest (24%) in selected farms. Except for those older than 66 months, the
188 ratios of the other age ranges were higher on the selected farms. In the carcass weight
189 distribution of the total slaughtered cows in 2023, the 'less than 400 kg' carcass was
190 predominant, accounting for 74% of the carcass weight distribution. The '400 ~ 500 kg'
191 accounted for 25%, and only 1% was the 'over 500 kg'. However, the carcass weight
192 distribution of the slaughtered cows from selected farm showed 57%, 39%, and 4% of the
193 'less than 400 kg', '400 ~ 500 kg', and 'over 500 kg,' respectively. These results indicate that
194 the slaughtered cows from the selected farms obtained higher quality grades and heavier

195 carcass weights than the total number of slaughtered cows in Korea, although the cows were
196 younger. A similar result, except for age, was found by Choi et al. (2017), who reported an
197 increase in the quality grade of boiled feed without differences in meat quality.

198 Representative images of the cut surface, loin-eye area, *spinalis thoracis* muscle
199 area, intermuscular fat area, and fat-to-lean ratio of the loin between the 7th and 8th *thoracic*
200 *vertebrae* are shown in Fig. 2. The fat-to-lean ratio tended to be lower in the TR group than in
201 the CON group because of the larger *spinalis thoracis* muscle and smaller intermuscular fat
202 area; however, the difference was not significant ($P > 0.05$). Muscle size, especially the loin-
203 eye area, has been shown to be related to carcass characteristics such as length or
204 circumference in previous studies; however, it seems that similar grades led to no significant
205 differences in the present study (Cole et al., 1960).

206 Representative stained images obtained using immunohistochemistry and muscle
207 fiber characteristics are shown in Fig. 3. The cross-sectional area, relative fiber number and
208 area, and density of each fiber were not significantly different ($P > 0.05$). Considering that a
209 previous study reported changes in muscle fiber characteristics by feeding regime in young
210 bulls with a live weight under 460 kg, these results indicate that different feeding regimes do
211 not lead to changes in the muscle fiber characteristics of cows whose growth is finished
212 (Vestergaard et al., 2000).

213 A Comparison of beef strip loin meat quality traits obtained from cows fed the
214 Korean Feeding Standard for Hanwoo cattle and boiled feed is shown in Fig. 4. Proximate
215 composition, lightness, pH, drip loss, aerobic microbial counts, and shear force did not show
216 significant differences between the two group of beef strip loin from cow ($P > 0.05$). Purge
217 loss was higher in the CON group than in the TR group during aging, and redness and
218 yellowness after aging were lower in the TR group than in the CON group ($P < 0.05$).

219 Similarly, cooking loss was lower in the TR group after aging ($P < 0.05$). The effect of aging
220 was greater in the CON group, as lightness, redness, yellowness, pH, cooking loss, and
221 aerobic microbial count increased after aging ($P < 0.05$). Beef strip loins from different
222 feeding regime groups showed decreased shear force after aging ($P < 0.05$). It seems that the
223 color changes during aging are caused by the effect of mineral content in the boiled feed,
224 such as iron and manganese, just as a previous study reported that beef strip loins fed with
225 minerals such as copper, manganese, and zinc, showed significantly lower redness than a
226 group where minerals were not added during storage (Harsh et al., 2018). In addition, Rossi
227 et al. (2020) reported that a higher concentration of mineral content in supplements leads to
228 lower redness and yellowness; therefore, feeding high concentrations of mineral content
229 results in color stability differences during storage. Not only meat color, but water-holding
230 capacity also seems to be affected by feed. As reported in previous study where the inclusion
231 of conjugated linoleic acid in the feed affected the water-holding capacity of meat, it seems
232 that the composition or type of feed changes the characteristics of skeletal muscle and
233 intramuscular fat, thereby leading to changes in fatty acid composition, meat color, and
234 water-holding capacity.

235 The fatty acid compositions of beef strip loin obtained from the Korean feeding
236 standard for Hanwoo (NIAS, 2022) and boiled feed-fed cows are shown in Table 3. The total
237 fatty acid content showed no significant difference between the treatments ($P > 0.05$). Many
238 fatty acids, including octanoic acid (C8:0), decanoic acid (C10:0), and dodecanoic acid
239 (C12:0), showed significant differences between the treatments and aging, and TR showed
240 higher levels of unsaturated fatty acids and lower levels of saturated fatty acids ($P < 0.05$). In
241 addition, TR was higher in both mono- and polyunsaturated fatty acids, regardless of the
242 storage day ($P < 0.05$). Since a previous study discovered that saturated fatty acids are related
243 to an increase in low-density lipoproteins, research interest has focused on fatty acids in food,

244 and researchers have attempted to control the fatty acid composition of beef as well (Vahmani
245 et al., 2015). For example, beef fatty acids are manipulated by breed, feeding regime, and
246 feed. A previous study reported increased omega-3 fatty acids by feeding on forage during the
247 finishing period (De Freitas et al., 2014). Ku et al. (2020) reported a decreased n-6/n-3 ratio
248 in Hanwoo steers due to higher forage feed. Furthermore, Kim et al. (2021) reported
249 increased unsaturated fatty acid content in boiled steer beef. In the previous study, the overall
250 expected degradability of neutral detergent fiber by rumen microbes was increased and
251 tended to increase over 60 hours of the *in situ* digestion model (Zhang et al., 2017). In other
252 words, this suggests that the less digestible cellulose in forage is weakened by the boiling
253 procedure, possibly resulting in beef with high unsaturated fatty acid content due to increased
254 fiber digestion and absorption.

255 The amino acid compositions of the two cow beef strip loins are shown in Table 4.
256 No significant differences were found between the treatments ($P > 0.05$). Amino acids are the
257 basic units of proteins and are a major reason for meat consumption (Oh et al., 2016).
258 Previous studies reported effect of gender on amino acids, but little effect of breed and
259 carcass weight (Holló et al., 2001). Only a few studies have focused on the effect of feed on
260 amino acids; Skelley et al. (1978) reported less change in amino acid composition than in
261 fatty acid composition, supporting the present result that the effect of feed on beef amino acid
262 composition is very little.

263

264 **4. Conclusion**

265 Taken together, the characteristics of muscle and muscle fibers between common
266 and boiled cow beef strip loin were not different, but some traits, including color, water-
267 holding capacity, and fatty acid composition, were different. Considering that cows from the

268 boiled feed group graded higher in the carcass quality grading (higher than 1 grade), these
269 meat quality traits are advantageous in regard to the higher unsaturated and lower saturated
270 fatty acid ratios of the cow beef fed with boiled feed, which can be accepted positively by
271 consumers when compared to common cow beef. In addition, from the perspective of
272 producers, it is thought that boiled feeding is a way to maximize productivity by not only
273 lowering production costs using low-cost sources and agricultural by-products, but also
274 raising profit by upgrading carcass grade.

275

ACCEPTED

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339

340 **Figure legends**

341 **Fig. 1. Number, carcass quality grade, age, and carcass weight of beef cow slaughtered**
342 **in 2023, Korea.** Statistics were obtained from KAPE (2025).

343 **Fig. 2. Representative images of the cut surface (A) and comparison of loin-eye area, M.**
344 ***spinalis thoracis* area, intramuscular fat area, and ratio of fat to lean (B) obtained from**
345 **beef cow loin between 7th and 8th thoracic vertebra.** CON, cow fed with Korean Feeding
346 Standard for Hanwoo (NIAS, 2022); TR, cow fed with boiled feed; InterMF, intermuscular
347 fat; LT, *longissimus thoracis* muscle; ST, *spinalis thoracis* muscle.

348 **Fig. 3. Representative images stained by immunohistochemistry (A) and result of muscle**
349 **fiber characteristics (cross-sectional area, relative fiber number and area, and fiber**
350 **density; B).** CON, cow fed with Korean Feeding Standard for Hanwoo (NIAS, 2022); TR,
351 cow fed with boiled feed.

352 **Fig. 4. Comparison of the proximate composition and meat quality properties of beef**
353 **strip loin (*M. longissimus lumborum*).** CON, cow fed with Korean Feeding Standard for
354 Hanwoo (NIAS, 2022); TR, cow fed with boiled feed. Different letters on the bar indicate
355 significant differences between the cows with different feeding within same storage day (^{a-b})
356 or between the different storage day within same feeding (^{x-y}).

357

358 Table 1. Formula of feed ingredient for beef cow.

	Contents	CON	TR
Feed ingredient (%)	Concentrate mix	68	6.3
	Lupin	-	14.4
	Soybean	-	11.9
	Corn grain	-	35.8
	Soybean hulls	-	4.3
	Corn hulls	-	2.3
	Rice bran	-	9.3
	Rice straw	32	15.6
	Salt	-	0.039
	Total	100	100
Feed nutrient	DM (%)	89.93	62.17
	Crude protein (% DM)	14.58	16.60
	Sol-CP (% DM)	4.36	7.10
	ADICP (% DM)	1.55	1.31
	NDICP (% DM)	3.21	2.17
	ADF (% DM)	20.80	20.3
	NDF (% DM)	40.21	36.00
	Lignin (% DM)	4.48	4.65
	NFC (% DM)	37.32	33.93
	Starch (% DM)	29.89	22.40
	Crude fat (% DM)	2.89	6.67
	Crude ash (% DM)	8.21	8.97
	Calcium (% DM)	0.90	0.59
	Phosphorus (% DM)	0.41	0.58
	Magnesium (% DM)	0.26	0.33
	Potassium (% DM)	0.82	1.09
	Sulfur (% DM)	0.30	0.25
	Sodium (ppm)	0.54	0.24
	Chloride (ppm)	0.63	0.46
	Iron (ppm)	636.49	928
Manganese (ppm)	128.23	4441	
Zinc (ppm)	105.26	102.00	
TDN	65.32	70.20	

CON, cow fed with Korean Feeding Standard for Hanwoo (NIAS, 2022); TR, cow fed with boiled feed; DM, dry matter; CP, crude protein, Sol-CP, soluble CP; ADICP, acid detergent insoluble CP; NDICP, neutral detergent insoluble CP; ADF, acid detergent fiber; NDF, neutral detergent fiber; NFC, non-fiber carbohydrate; TDN, total digestible nutrient.

360 Table 2. Comparison of fatty acid compositions between the cows with different types of feed.

Fatty acids (%)	Day 2		Day 16		PSE	Level of significance		
	CON	TR	CON	TR		TR	SD	TR × SD
Total fatty acids (mg/g IMF)	88.93	87.07	102.84	115.44	8.49	ns	ns	ns
C6:0	0.02 ^b	0.05 ^a	0.03 ^{ab}	0.03 ^{ab}	0.00	ns	ns	*
C8:0	0.01 ^b	0.03 ^a	0.01 ^b	0.01 ^b	0.00	**	*	**
C10:0	0.07	0.08	0.06	0.07	0.00	ns	***	ns
C12:0	0.12	0.10	0.10	0.09	0.00	*	*	ns
C14:0	3.42	2.99	3.40	3.01	0.07	***	ns	ns
C14:1	1.07	0.84	1.09	0.81	0.05	ns	ns	ns
C15:0	0.25	0.31	0.25	0.31	0.01	ns	ns	ns
C16:0	28.21	24.98	28.12	25.55	0.32	***	ns	ns
C16:1	5.47	4.95	5.67	4.97	0.12	ns	ns	ns
C17:0	0.65	0.84	0.65	0.85	0.03	ns	ns	ns
C17:1	0.62	0.79	0.61	0.78	0.02	ns	ns	ns
C18:0	10.75	9.65	10.62	9.90	0.26	***	ns	ns
C18:1 n9 <i>Cis</i>	0.55	1.53	0.33	0.86	0.09	*	**	ns
C18:1 n9 <i>Trans</i>	45.64	48.26	46.06	48.32	0.39	***	*	ns
C18:2 n6 <i>Cis</i>	0.15	0.18	0.16	0.17	0.01	ns	ns	ns
C18:2 n6 <i>Trans</i>	1.66	2.71	1.54	2.74	0.10	***	ns	ns
C18:3 n6	0.08	0.08	0.08	0.08	0.00	*	ns	ns
C18:3 n3	0.05 ^b	0.33 ^a	0.03 ^b	0.05 ^b	0.03	**	**	**
C20:0	0.26 ^b	0.18 ^c	0.27 ^b	0.37 ^a	0.02	ns	**	*
C20:1 n9	0.09 ^{bc}	0.41 ^a	0.05 ^c	0.12 ^b	0.03	***	***	***
C20:2	0.25	0.19	0.31	0.39	0.02	ns	**	ns
C20:3 n6	0.08	0.07	0.11	0.18	0.01	ns	*	ns
C20:3 n3	0.07	0.11	0.06	0.04	0.01	ns	ns	ns
C20:4 n6	0.10	0.06	0.12	0.10	0.01	*	*	ns
C20:5 n3	0.03	<i>n. d.</i>	0.02	0.03	0.00	ns	ns	ns
C21:0	0.02	0.03	0.01	0.02	0.00	ns	ns	ns
C22:0	0.01 ^b	0.02 ^a	0.01 ^b	0.01 ^{ab}	0.00	ns	ns	*
C22:1 n9	0.22	0.11	0.15	0.09	0.01	*	ns	ns
C22:2	0.02	0.04	0.02	0.02	0.00	ns	ns	ns
C22:6 n3	0.02	0.04	0.01	0.02	0.00	ns	ns	ns
C23:0	0.04	0.03	0.01	0.02	0.01	ns	ns	ns
C24:0	0.03	0.08	0.02	0.04	0.01	ns	ns	ns
SFA	43.84	39.33	43.57	40.25	0.50	***	ns	ns
UFA	56.16	60.67	56.43	59.75	0.50	***	ns	ns
MUFA	53.67	56.89	53.96	55.96	0.44	***	ns	ns
PUFA	2.49	3.78	2.47	3.79	0.12	***	ns	ns
∑n3	0.15 ^b	0.49 ^a	0.13 ^b	0.11 ^b	0.03	ns	**	**
∑n6	2.07	3.08	2.01	3.27	0.11	ns	ns	ns

Σ n9	46.51	50.32	46.59	49.40	0.42	***	ns	ns
n3:n6	0.07 ^b	0.16 ^a	0.07 ^b	0.03 ^c	0.01	**	***	***

Data are means and pooled standard error (PSE).

^{a-c}Means with different letters indicate significant difference ($P < 0.05$)

CON, cow fed with Korean Feeding Standard for Hanwoo (NIAS, 2022); TR, cow fed with boiled feed; IMF, intramuscular fat; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Σ n3, sum of n3 fatty acids; Σ n6, sum of n6 fatty acids; Σ n9, sum of n9 fatty acids.

ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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362 Table 3. Comparison of amino acid composition between the beef cows with different types of
 363 feed.

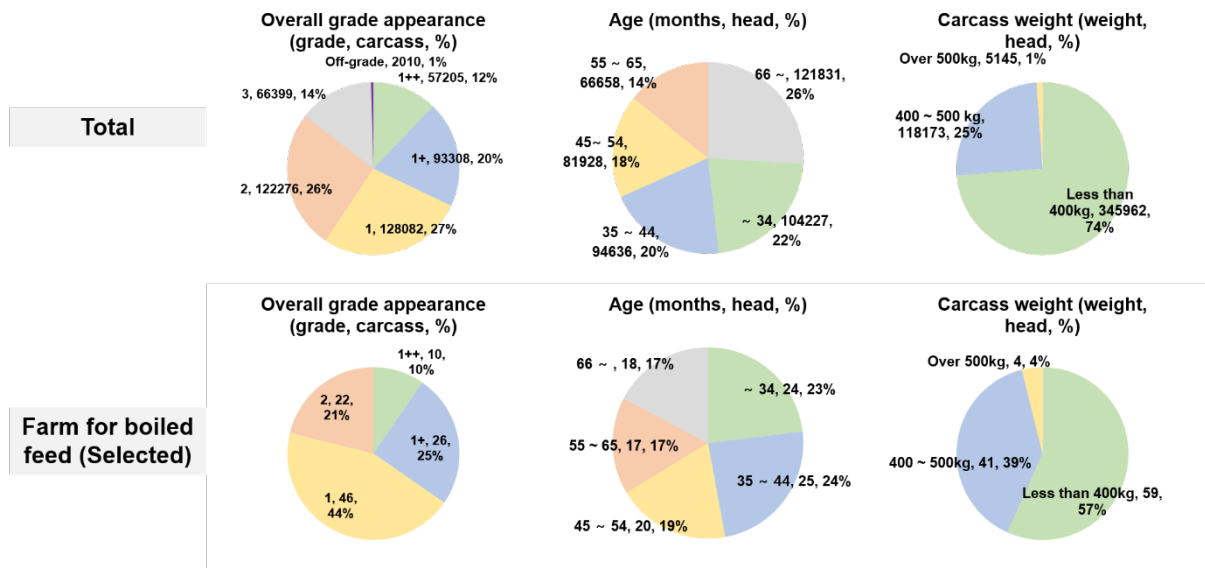
Amino acids (%)	CON	TR	PSE	Level of significance
Aspartic acid	5.16	4.97	0.10	ns
Glutamic acid	15.65	15.84	0.25	ns
Serine	4.20	4.31	0.05	ns
Histidine	6.31	6.60	0.31	ns
Glycine	7.83	8.12	0.31	ns
Threonine	4.79	4.63	0.07	ns
Arginine	12.95	12.85	0.16	ns
Alanine	6.86	6.87	0.05	ns
Tyrosine	3.73	3.79	0.04	ns
Valine	4.54	4.32	0.08	ns
Methionine	2.93	3.04	0.08	ns
Phenylalanine	4.21	4.11	0.07	ns
Isoleucine	4.63	4.40	0.10	ns
Leucine	10.27	10.26	0.17	ns
Lysine	5.94	5.90	0.20	ns

CON, cow fed with Korean Feeding Standard for Hanwoo (NIAS, 2022); TR, cow fed with boiled feed; ns, not significant.

364

365

366 **Fig. 1.**

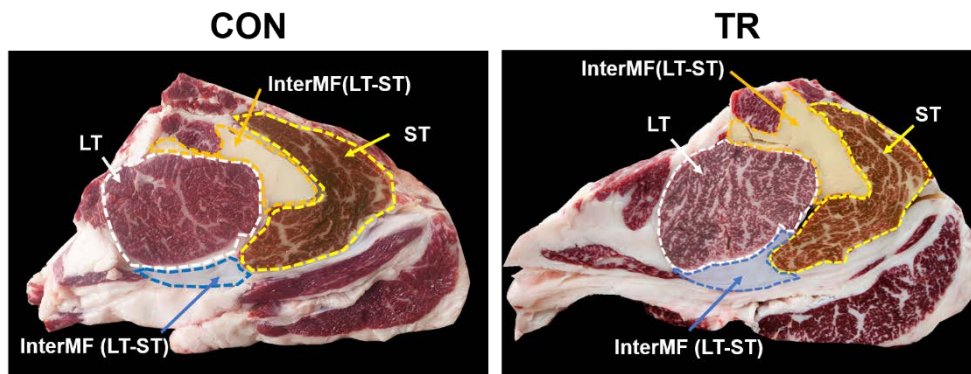


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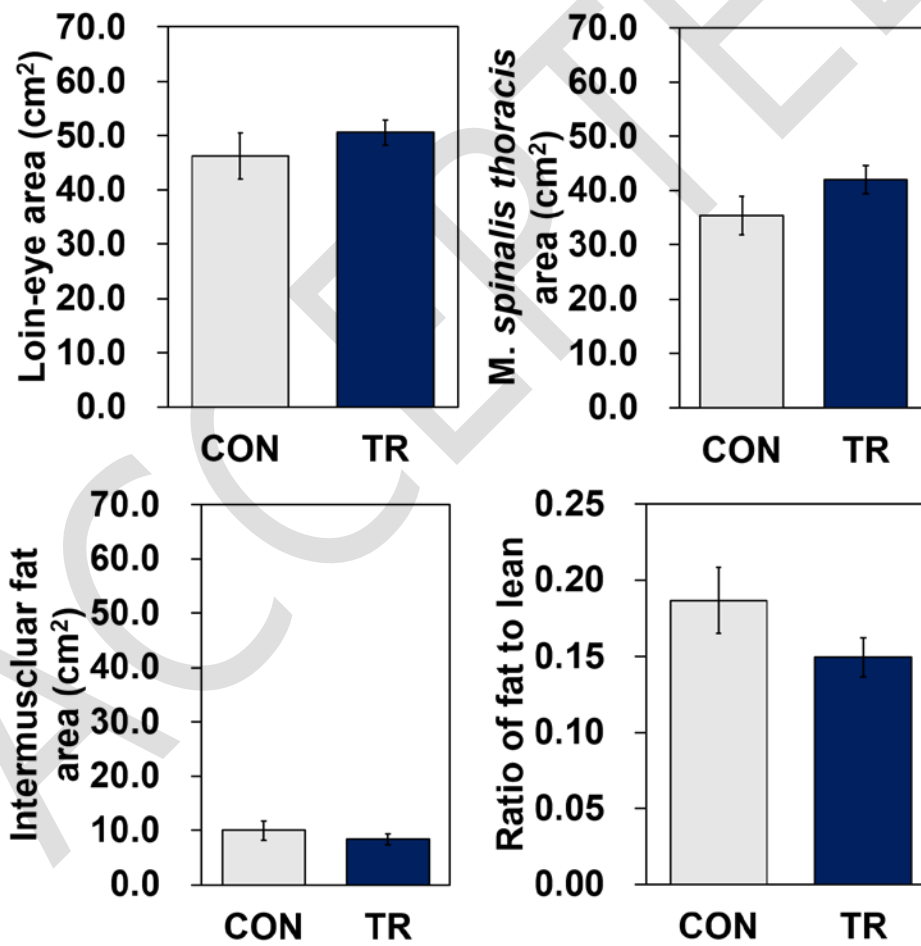
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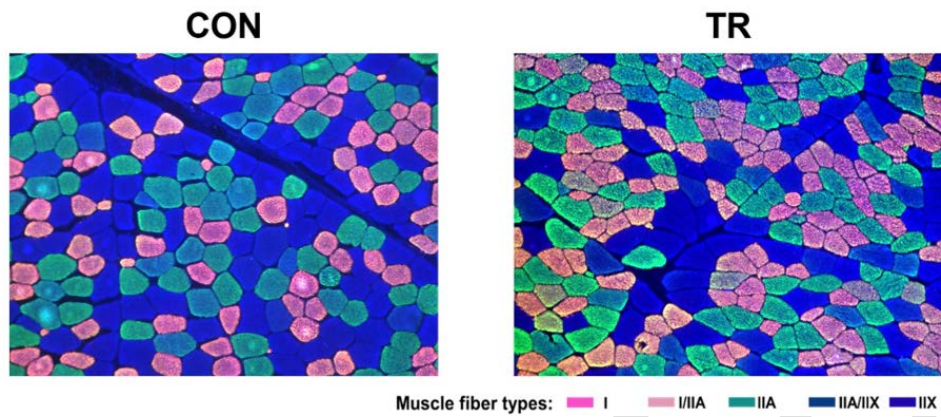
A



B



A



B

